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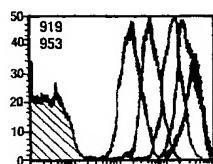
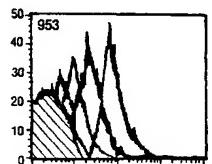
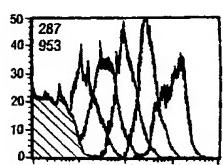
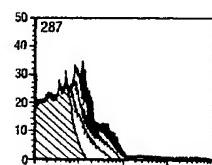
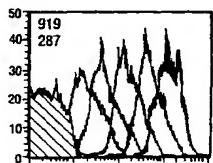
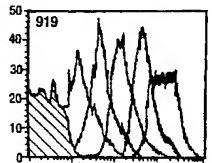
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[Continued on next page]

(54) Title: COMBINATION NEISSERIAL COMPOSITIONS



- pre-immune
- 1 1:800
- 1 1:2400
- 1 1:7200
- 1 1:21600
- 1 1:64800

(57) Abstract: Compositions comprising a first biological molecule from a *Neisseria* bacterium and a second biological molecule from a *Neiseria* bacterium. The term "biological molecule" includes proteins and nucleic acids. Preferred *Neisseria* species are *N.meningitidis* and *N.gonorrhoeae*

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COMBINATION NEISSERIAL COMPOSITIONS

All documents cited herein are incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to compositions comprising combinations of biological molecules from 5 *Neisseria* bacteria, particularly *N.meningitidis* and *N.gonorrhoeae*.

BACKGROUND ART

Neisseria meningitidis and *Neisseria gonorrhoeae* are non-motile, Gram negative diplococci that are pathogenic in humans.

Based on the organism's capsular polysaccharide, 12 serogroups of *N.meningitidis* have been 10 identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries.

The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed 15 of serogroups A, C, Y and W135. Meningococcus B remains a problem, however. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of $\alpha(2-8)$ -linked N-acetyl neuraminic acid that is also present in mammalian tissue. One approach to a menB vaccine uses mixtures of outer membrane proteins (OMPs) To 20 overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed [eg. Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28]. Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability [eg. Ala-Aldeen & Borriello (1996) The meningococcal transferrin-binding 25 proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53].

Given the propensity for meningococcal disease during non-epidemic periods to be caused by multiple strains or strain variants [Russel *et al.* (1998) *Abstracts of 11th International pathogenic Neisseria conference*, page 281] together with frequent temporal shifts in the predominant strains in a community, it seems that a universal meningococcal B vaccine will 30 require more than one antigenic species.

DESCRIPTION OF THE INVENTION

Neisserial protein and nucleotide sequences are disclosed in the following documents:

- WO 99/24578
- WO 99/36544
- WO 99/57280
- WO 97/28273
- WO 96/29412
- WO 95/03413
- Tettelin *et al.* (2000) *Science* 287:1809-1815

- 5 10 For ease of reference, the sequences disclosed in these documents are referred to in the present application according to the following table:

Document	Original sequence numbering	Sequence numbering in this application
WO 99/24578	SEQ IDs 1-892	SEQ IDs 1-892
WO 99/36544	SEQ IDs 1-90	SEQ IDs 893-982
WO 99/57280	SEQ IDs 1-3020	SEQ IDs 983-4002
WO 97/28273	Figure 4 coding DNA Figure 4 protein Figure 9 coding DNA Figure 13 DNA Figure 13 protein	SEQ ID 4003 SEQ ID 4004 SEQ ID 4005 SEQ ID 4006 SEQ ID 4007
WO 96/29412	SEQ IDs 1-26	SEQ IDs 4008-4033
WO 95/03413	SEQ IDs 1-23	SEQ IDs 4034-4056
Tettelin <i>et al.</i> (2000) <i>Science</i> 287:1809-15	NMB0001-2160 (DNA) NMB0001-2160 (encoded proteins)	SEQ IDs 4057-6216 SEQ IDs 6217-8376

The present invention provides compositions comprising a first biological molecule from a *Neisseria* bacterium and a second biological molecule from a *Neisseria* bacterium. The term "biological molecule" includes proteins and nucleic acids.

- 15 15 The compositions may also comprise further biological molecules, preferably also from *Neisseria*, that is to say the compositions may comprise two or more biological molecules (eg. 3, 4, 5, 6, 7, 8 etc.), at least two of which are from a *Neisseria* bacterium (eg. 3, 4, 5, 6, 7, 8 etc.). Such compositions include those comprising (i) two or more different Neisserial proteins.

(ii) two or more different Neisserial nucleic acids, or (iii) mixtures of one or more Neisserial protein and one or more Neisserial nucleic acid.

In one preferred embodiment, the first and second biological molecules are from different *Neisseria* species (eg. one is from *N.meningitidis* and one is from *N.gonorrhoeae*), but they may be from the same species. The biological molecules in the compositions may be from different serogroups or strains of the same species.

The first biological molecule is preferably selected from the group consisting of SEQ IDs 1-8376. More preferably, it is selected from the group consisting of SEQ IDs 1-4002 and/or SEQ IDs 4057-8376. It is preferably a purified or isolated biological molecule.

10 The second biological molecule is preferably selected from the group consisting of SEQ IDs 1-8376. More preferably, it is selected from the group consisting of SEQ IDs 1-4002 and/or SEQ IDs 4057-8376. It is preferably a purified or isolated biological molecule.

One or both of the first and second biological molecules may be a Neisserial biological molecule which is not specifically disclosed herein, and which may not have been identified, 15 discovered, made available to the public or purified before this patent application was filed.

In particular, the invention provides a composition comprising one or more of the following pairs of first and second biological molecules (listed by SEQ ID):

First	Second	24	1-23 and 25-8376	48	1-47 and 49-8376
1	2-8376	25	1-24 and 26-8376	49	1-48 and 50-8376
2	1 and 3-8376	26	1-25 and 27-8376	50	1-49 and 51-8376
3	1-2 and 4-8376	27	1-26 and 28-8376	51	1-50 and 52-8376
4	1-3 and 5-8376	28	1-27 and 29-8376	52	1-51 and 53-8376
5	1-4 and 6-8376	29	1-28 and 30-8376	53	1-52 and 54-8376
6	1-5 and 7-8376	30	1-29 and 31-8376	54	1-53 and 55-8376
7	1-6 and 8-8376	31	1-30 and 32-8376	55	1-54 and 56-8376
8	1-7 and 9-8376	32	1-31 and 33-8376	56	1-55 and 57-8376
9	1-8 and 10-8376	33	1-32 and 34-8376	57	1-56 and 58-8376
10	1-9 and 11-8376	34	1-33 and 35-8376	58	1-57 and 59-8376
11	1-10 and 12-8376	35	1-34 and 36-8376	59	1-58 and 60-8376
12	1-11 and 13-8376	36	1-35 and 37-8376	60	1-59 and 61-8376
13	1-12 and 14-8376	37	1-36 and 38-8376	61	1-60 and 62-8376
14	1-13 and 15-8376	38	1-37 and 39-8376	62	1-61 and 63-8376
15	1-14 and 16-8376	39	1-38 and 40-8376	63	1-62 and 64-8376
16	1-15 and 17-8376	40	1-39 and 41-8376	64	1-63 and 65-8376
17	1-16 and 18-8376	41	1-40 and 42-8376	65	1-64 and 66-8376
18	1-17 and 19-8376	42	1-41 and 43-8376	66	1-65 and 67-8376
19	1-18 and 20-8376	43	1-42 and 44-8376	67	1-66 and 68-8376
20	1-19 and 21-8376	44	1-43 and 45-8376	68	1-67 and 69-8376
21	1-20 and 22-8376	45	1-44 and 46-8376	69	1-68 and 70-8376
22	1-21 and 23-8376	46	1-45 and 47-8376	70	1-69 and 71-8376
23	1-22 and 24-8376	47	1-46 and 48-8376	71	1-70 and 72-8376

72	1-71 and 73-8376	129	1-128 and 130-8376	186	1-185 and 187-8376
73	1-72 and 74-8376	130	1-129 and 131-8376	187	1-186 and 188-8376
74	1-73 and 75-8376	131	1-130 and 132-8376	188	1-187 and 189-8376
75	1-74 and 76-8376	132	1-131 and 133-8376	189	1-188 and 190-8376
76	1-75 and 77-8376	133	1-132 and 134-8376	190	1-189 and 191-8376
77	1-76 and 78-8376	134	1-133 and 135-8376	191	1-190 and 192-8376
78	1-77 and 79-8376	135	1-134 and 136-8376	192	1-191 and 193-8376
79	1-78 and 80-8376	136	1-135 and 137-8376	193	1-192 and 194-8376
80	1-79 and 81-8376	137	1-136 and 138-8376	194	1-193 and 195-8376
81	1-80 and 82-8376	138	1-137 and 139-8376	195	1-194 and 196-8376
82	1-81 and 83-8376	139	1-138 and 140-8376	196	1-195 and 197-8376
83	1-82 and 84-8376	140	1-139 and 141-8376	197	1-196 and 198-8376
84	1-83 and 85-8376	141	1-140 and 142-8376	198	1-197 and 199-8376
85	1-84 and 86-8376	142	1-141 and 143-8376	199	1-198 and 200-8376
86	1-85 and 87-8376	143	1-142 and 144-8376	200	1-199 and 201-8376
87	1-86 and 88-8376	144	1-143 and 145-8376	201	1-200 and 202-8376
88	1-87 and 89-8376	145	1-144 and 146-8376	202	1-201 and 203-8376
89	1-88 and 90-8376	146	1-145 and 147-8376	203	1-202 and 204-8376
90	1-89 and 91-8376	147	1-146 and 148-8376	204	1-203 and 205-8376
91	1-90 and 92-8376	148	1-147 and 149-8376	205	1-204 and 206-8376
92	1-91 and 93-8376	149	1-148 and 150-8376	206	1-205 and 207-8376
93	1-92 and 94-8376	150	1-149 and 151-8376	207	1-206 and 208-8376
94	1-93 and 95-8376	151	1-150 and 152-8376	208	1-207 and 209-8376
95	1-94 and 96-8376	152	1-151 and 153-8376	209	1-208 and 210-8376
96	1-95 and 97-8376	153	1-152 and 154-8376	210	1-209 and 211-8376
97	1-96 and 98-8376	154	1-153 and 155-8376	211	1-210 and 212-8376
98	1-97 and 99-8376	155	1-154 and 156-8376	212	1-211 and 213-8376
99	1-98 and 100-8376	156	1-155 and 157-8376	213	1-212 and 214-8376
100	1-99 and 101-8376	157	1-156 and 158-8376	214	1-213 and 215-8376
101	1-100 and 102-8376	158	1-157 and 159-8376	215	1-214 and 216-8376
102	1-101 and 103-8376	159	1-158 and 160-8376	216	1-215 and 217-8376
103	1-102 and 104-8376	160	1-159 and 161-8376	217	1-216 and 218-8376
104	1-103 and 105-8376	161	1-160 and 162-8376	218	1-217 and 219-8376
105	1-104 and 106-8376	162	1-161 and 163-8376	219	1-218 and 220-8376
106	1-105 and 107-8376	163	1-162 and 164-8376	220	1-219 and 221-8376
107	1-106 and 108-8376	164	1-163 and 165-8376	221	1-220 and 222-8376
108	1-107 and 109-8376	165	1-164 and 166-8376	222	1-221 and 223-8376
109	1-108 and 110-8376	166	1-165 and 167-8376	223	1-222 and 224-8376
110	1-109 and 111-8376	167	1-166 and 168-8376	224	1-223 and 225-8376
111	1-110 and 112-8376	168	1-167 and 169-8376	225	1-224 and 226-8376
112	1-111 and 113-8376	169	1-168 and 170-8376	226	1-225 and 227-8376
113	1-112 and 114-8376	170	1-169 and 171-8376	227	1-226 and 228-8376
114	1-113 and 115-8376	171	1-170 and 172-8376	228	1-227 and 229-8376
115	1-114 and 116-8376	172	1-171 and 173-8376	229	1-228 and 230-8376
116	1-115 and 117-8376	173	1-172 and 174-8376	230	1-229 and 231-8376
117	1-116 and 118-8376	174	1-173 and 175-8376	231	1-230 and 232-8376
118	1-117 and 119-8376	175	1-174 and 176-8376	232	1-231 and 233-8376
119	1-118 and 120-8376	176	1-175 and 177-8376	233	1-232 and 234-8376
120	1-119 and 121-8376	177	1-176 and 178-8376	234	1-233 and 235-8376
121	1-120 and 122-8376	178	1-177 and 179-8376	235	1-234 and 236-8376
122	1-121 and 123-8376	179	1-178 and 180-8376	236	1-235 and 237-8376
123	1-122 and 124-8376	180	1-179 and 181-8376	237	1-236 and 238-8376
124	1-123 and 125-8376	181	1-180 and 182-8376	238	1-237 and 239-8376
125	1-124 and 126-8376	182	1-181 and 183-8376	239	1-238 and 240-8376
126	1-125 and 127-8376	183	1-182 and 184-8376	240	1-239 and 241-8376
127	1-126 and 128-8376	184	1-183 and 185-8376	241	1-240 and 242-8376
128	1-127 and 129-8376	185	1-184 and 186-8376	242	1-241 and 243-8376

243	I-242 and 244-8376	300	I-299 and 301-8376	357	I-356 and 358-8376
244	I-243 and 245-8376	301	I-300 and 302-8376	358	I-357 and 359-8376
245	I-244 and 246-8376	302	I-301 and 303-8376	359	I-358 and 360-8376
246	I-245 and 247-8376	303	I-302 and 304-8376	360	I-359 and 361-8376
247	I-246 and 248-8376	304	I-303 and 305-8376	361	I-360 and 362-8376
248	I-247 and 249-8376	305	I-304 and 306-8376	362	I-361 and 363-8376
249	I-248 and 250-8376	306	I-305 and 307-8376	363	I-362 and 364-8376
250	I-249 and 251-8376	307	I-306 and 308-8376	364	I-363 and 365-8376
251	I-250 and 252-8376	308	I-307 and 309-8376	365	I-364 and 366-8376
252	I-251 and 253-8376	309	I-308 and 310-8376	366	I-365 and 367-8376
253	I-252 and 254-8376	310	I-309 and 311-8376	367	I-366 and 368-8376
254	I-253 and 255-8376	311	I-310 and 312-8376	368	I-367 and 369-8376
255	I-254 and 256-8376	312	I-311 and 313-8376	369	I-368 and 370-8376
256	I-255 and 257-8376	313	I-312 and 314-8376	370	I-369 and 371-8376
257	I-256 and 258-8376	314	I-313 and 315-8376	371	I-370 and 372-8376
258	I-257 and 259-8376	315	I-314 and 316-8376	372	I-371 and 373-8376
259	I-258 and 260-8376	316	I-315 and 317-8376	373	I-372 and 374-8376
260	I-259 and 261-8376	317	I-316 and 318-8376	374	I-373 and 375-8376
261	I-260 and 262-8376	318	I-317 and 319-8376	375	I-374 and 376-8376
262	I-261 and 263-8376	319	I-318 and 320-8376	376	I-375 and 377-8376
263	I-262 and 264-8376	320	I-319 and 321-8376	377	I-376 and 378-8376
264	I-263 and 265-8376	321	I-320 and 322-8376	378	I-377 and 379-8376
265	I-264 and 266-8376	322	I-321 and 323-8376	379	I-378 and 380-8376
266	I-265 and 267-8376	323	I-322 and 324-8376	380	I-379 and 381-8376
267	I-266 and 268-8376	324	I-323 and 325-8376	381	I-380 and 382-8376
268	I-267 and 269-8376	325	I-324 and 326-8376	382	I-381 and 383-8376
269	I-268 and 270-8376	326	I-325 and 327-8376	383	I-382 and 384-8376
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271	I-270 and 272-8376	328	I-327 and 329-8376	385	I-384 and 386-8376
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424	1-423 and 425-8376	481	1-480 and 482-8376	538	1-537 and 539-8376
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1838	I-1837 and 1839-8376	1895	I-1894 and 1896-8376	1952	I-1951 and 1953-8376

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1954	I-1953 and 1955-8376	2011	I-2010 and 2012-8376	2068	I-2067 and 2069-8376
1955	I-1954 and 1956-8376	2012	I-2011 and 2013-8376	2069	I-2068 and 2070-8376
1956	I-1955 and 1957-8376	2013	I-2012 and 2014-8376	2070	I-2069 and 2071-8376
1957	I-1956 and 1958-8376	2014	I-2013 and 2015-8376	2071	I-2070 and 2072-8376
1958	I-1957 and 1959-8376	2015	I-2014 and 2016-8376	2072	I-2071 and 2073-8376
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1963	I-1962 and 1964-8376	2020	I-2019 and 2021-8376	2077	I-2076 and 2078-8376
1964	I-1963 and 1965-8376	2021	I-2020 and 2022-8376	2078	I-2077 and 2079-8376
1965	I-1964 and 1966-8376	2022	I-2021 and 2023-8376	2079	I-2078 and 2080-8376
1966	I-1965 and 1967-8376	2023	I-2022 and 2024-8376	2080	I-2079 and 2081-8376
1967	I-1966 and 1968-8376	2024	I-2023 and 2025-8376	2081	I-2080 and 2082-8376
1968	I-1967 and 1969-8376	2025	I-2024 and 2026-8376	2082	I-2081 and 2083-8376
1969	I-1968 and 1970-8376	2026	I-2025 and 2027-8376	2083	I-2082 and 2084-8376
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1971	I-1970 and 1972-8376	2028	I-2027 and 2029-8376	2085	I-2084 and 2086-8376
1972	I-1971 and 1973-8376	2029	I-2028 and 2030-8376	2086	I-2085 and 2087-8376
1973	I-1972 and 1974-8376	2030	I-2029 and 2031-8376	2087	I-2086 and 2088-8376
1974	I-1973 and 1975-8376	2031	I-2030 and 2032-8376	2088	I-2087 and 2089-8376
1975	I-1974 and 1976-8376	2032	I-2031 and 2033-8376	2089	I-2088 and 2090-8376
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1985	I-1984 and 1986-8376	2042	I-2041 and 2043-8376	2099	I-2098 and 2100-8376
1986	I-1985 and 1987-8376	2043	I-2042 and 2044-8376	2100	I-2099 and 2101-8376
1987	I-1986 and 1988-8376	2044	I-2043 and 2045-8376	2101	I-2100 and 2102-8376
1988	I-1987 and 1989-8376	2045	I-2044 and 2046-8376	2102	I-2101 and 2103-8376
1989	I-1988 and 1990-8376	2046	I-2045 and 2047-8376	2103	I-2102 and 2104-8376
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1991	I-1990 and 1992-8376	2048	I-2047 and 2049-8376	2105	I-2104 and 2106-8376
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1993	I-1992 and 1994-8376	2050	I-2049 and 2051-8376	2107	I-2106 and 2108-8376
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2125	1-2124 and 2126-8376	2182	1-2181 and 2183-8376	2239	1-2238 and 2240-8376
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2128	1-2127 and 2129-8376	2185	1-2184 and 2186-8376	2242	1-2241 and 2243-8376
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2132	1-2131 and 2133-8376	2189	1-2188 and 2190-8376	2246	1-2245 and 2247-8376
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2144	1-2143 and 2145-8376	2201	1-2200 and 2202-8376	2258	1-2257 and 2259-8376
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2165	1-2164 and 2166-8376	2222	1-2221 and 2223-8376	2279	1-2278 and 2280-8376
2166	1-2165 and 2167-8376	2223	1-2222 and 2224-8376	2280	1-2279 and 2281-8376
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7811	1-7810 and 7812-8376	7868	1-7867 and 7869-8376	7925	1-7924 and 7926-8376
7812	1-7811 and 7813-8376	7869	1-7868 and 7870-8376	7926	1-7925 and 7927-8376
7813	1-7812 and 7814-8376	7870	1-7869 and 7871-8376	7927	1-7926 and 7928-8376
7814	1-7813 and 7815-8376	7871	1-7870 and 7872-8376	7928	1-7927 and 7929-8376
7815	1-7814 and 7816-8376	7872	1-7871 and 7873-8376	7929	1-7928 and 7930-8376
7816	1-7815 and 7817-8376	7873	1-7872 and 7874-8376	7930	1-7929 and 7931-8376
7817	1-7816 and 7818-8376	7874	1-7873 and 7875-8376	7931	1-7930 and 7932-8376
7818	1-7817 and 7819-8376	7875	1-7874 and 7876-8376	7932	1-7931 and 7933-8376
7819	1-7818 and 7820-8376	7876	1-7875 and 7877-8376	7933	1-7932 and 7934-8376
7820	1-7819 and 7821-8376	7877	1-7876 and 7878-8376	7934	1-7933 and 7935-8376
7821	1-7820 and 7822-8376	7878	1-7877 and 7879-8376	7935	1-7934 and 7936-8376
7822	1-7821 and 7823-8376	7879	1-7878 and 7880-8376	7936	1-7935 and 7937-8376
7823	1-7822 and 7824-8376	7880	1-7879 and 7881-8376	7937	1-7936 and 7938-8376

7938	I-7937 and 7939-8376	7995	I-7994 and 7996-8376	8052	I-8051 and 8053-8376
7939	I-7938 and 7940-8376	7996	I-7995 and 7997-8376	8053	I-8052 and 8054-8376
7940	I-7939 and 7941-8376	7997	I-7996 and 7998-8376	8054	I-8053 and 8055-8376
7941	I-7940 and 7942-8376	7998	I-7997 and 7999-8376	8055	I-8054 and 8056-8376
7942	I-7941 and 7943-8376	7999	I-7998 and 8000-8376	8056	I-8055 and 8057-8376
7943	I-7942 and 7944-8376	8000	I-7999 and 8001-8376	8057	I-8056 and 8058-8376
7944	I-7943 and 7945-8376	8001	I-8000 and 8002-8376	8058	I-8057 and 8059-8376
7945	I-7944 and 7946-8376	8002	I-8001 and 8003-8376	8059	I-8058 and 8060-8376
7946	I-7945 and 7947-8376	8003	I-8002 and 8004-8376	8060	I-8059 and 8061-8376
7947	I-7946 and 7948-8376	8004	I-8003 and 8005-8376	8061	I-8060 and 8062-8376
7948	I-7947 and 7949-8376	8005	I-8004 and 8006-8376	8062	I-8061 and 8063-8376
7949	I-7948 and 7950-8376	8006	I-8005 and 8007-8376	8063	I-8062 and 8064-8376
7950	I-7949 and 7951-8376	8007	I-8006 and 8008-8376	8064	I-8063 and 8065-8376
7951	I-7950 and 7952-8376	8008	I-8007 and 8009-8376	8065	I-8064 and 8066-8376
7952	I-7951 and 7953-8376	8009	I-8008 and 8010-8376	8066	I-8065 and 8067-8376
7953	I-7952 and 7954-8376	8010	I-8009 and 8011-8376	8067	I-8066 and 8068-8376
7954	I-7953 and 7955-8376	8011	I-8010 and 8012-8376	8068	I-8067 and 8069-8376
7955	I-7954 and 7956-8376	8012	I-8011 and 8013-8376	8069	I-8068 and 8070-8376
7956	I-7955 and 7957-8376	8013	I-8012 and 8014-8376	8070	I-8069 and 8071-8376
7957	I-7956 and 7958-8376	8014	I-8013 and 8015-8376	8071	I-8070 and 8072-8376
7958	I-7957 and 7959-8376	8015	I-8014 and 8016-8376	8072	I-8071 and 8073-8376
7959	I-7958 and 7960-8376	8016	I-8015 and 8017-8376	8073	I-8072 and 8074-8376
7960	I-7959 and 7961-8376	8017	I-8016 and 8018-8376	8074	I-8073 and 8075-8376
7961	I-7960 and 7962-8376	8018	I-8017 and 8019-8376	8075	I-8074 and 8076-8376
7962	I-7961 and 7963-8376	8019	I-8018 and 8020-8376	8076	I-8075 and 8077-8376
7963	I-7962 and 7964-8376	8020	I-8019 and 8021-8376	8077	I-8076 and 8078-8376
7964	I-7963 and 7965-8376	8021	I-8020 and 8022-8376	8078	I-8077 and 8079-8376
7965	I-7964 and 7966-8376	8022	I-8021 and 8023-8376	8079	I-8078 and 8080-8376
7966	I-7965 and 7967-8376	8023	I-8022 and 8024-8376	8080	I-8079 and 8081-8376
7967	I-7966 and 7968-8376	8024	I-8023 and 8025-8376	8081	I-8080 and 8082-8376
7968	I-7967 and 7969-8376	8025	I-8024 and 8026-8376	8082	I-8081 and 8083-8376
7969	I-7968 and 7970-8376	8026	I-8025 and 8027-8376	8083	I-8082 and 8084-8376
7970	I-7969 and 7971-8376	8027	I-8026 and 8028-8376	8084	I-8083 and 8085-8376
7971	I-7970 and 7972-8376	8028	I-8027 and 8029-8376	8085	I-8084 and 8086-8376
7972	I-7971 and 7973-8376	8029	I-8028 and 8030-8376	8086	I-8085 and 8087-8376
7973	I-7972 and 7974-8376	8030	I-8029 and 8031-8376	8087	I-8086 and 8088-8376
7974	I-7973 and 7975-8376	8031	I-8030 and 8032-8376	8088	I-8087 and 8089-8376
7975	I-7974 and 7976-8376	8032	I-8031 and 8033-8376	8089	I-8088 and 8090-8376
7976	I-7975 and 7977-8376	8033	I-8032 and 8034-8376	8090	I-8089 and 8091-8376
7977	I-7976 and 7978-8376	8034	I-8033 and 8035-8376	8091	I-8090 and 8092-8376
7978	I-7977 and 7979-8376	8035	I-8034 and 8036-8376	8092	I-8091 and 8093-8376
7979	I-7978 and 7980-8376	8036	I-8035 and 8037-8376	8093	I-8092 and 8094-8376
7980	I-7979 and 7981-8376	8037	I-8036 and 8038-8376	8094	I-8093 and 8095-8376
7981	I-7980 and 7982-8376	8038	I-8037 and 8039-8376	8095	I-8094 and 8096-8376
7982	I-7981 and 7983-8376	8039	I-8038 and 8040-8376	8096	I-8095 and 8097-8376
7983	I-7982 and 7984-8376	8040	I-8039 and 8041-8376	8097	I-8096 and 8098-8376
7984	I-7983 and 7985-8376	8041	I-8040 and 8042-8376	8098	I-8097 and 8099-8376
7985	I-7984 and 7986-8376	8042	I-8041 and 8043-8376	8099	I-8098 and 8100-8376
7986	I-7985 and 7987-8376	8043	I-8042 and 8044-8376	8100	I-8099 and 8101-8376
7987	I-7986 and 7988-8376	8044	I-8043 and 8045-8376	8101	I-8100 and 8102-8376
7988	I-7987 and 7989-8376	8045	I-8044 and 8046-8376	8102	I-8101 and 8103-8376
7989	I-7988 and 7990-8376	8046	I-8045 and 8047-8376	8103	I-8102 and 8104-8376
7990	I-7989 and 7991-8376	8047	I-8046 and 8048-8376	8104	I-8103 and 8105-8376
7991	I-7990 and 7992-8376	8048	I-8047 and 8049-8376	8105	I-8104 and 8106-8376
7992	I-7991 and 7993-8376	8049	I-8048 and 8050-8376	8106	I-8105 and 8107-8376
7993	I-7992 and 7994-8376	8050	I-8049 and 8051-8376	8107	I-8106 and 8108-8376
7994	I-7993 and 7995-8376	8051	I-8050 and 8052-8376	8108	I-8107 and 8109-8376

8109	1-8108 and 8110-8376	8166	1-8165 and 8167-8376	8223	1-8222 and 8224-8376
8110	1-8109 and 8111-8376	8167	1-8166 and 8168-8376	8224	1-8223 and 8225-8376
8111	1-8110 and 8112-8376	8168	1-8167 and 8169-8376	8225	1-8224 and 8226-8376
8112	1-8111 and 8113-8376	8169	1-8168 and 8170-8376	8226	1-8225 and 8227-8376
8113	1-8112 and 8114-8376	8170	1-8169 and 8171-8376	8227	1-8226 and 8228-8376
8114	1-8113 and 8115-8376	8171	1-8170 and 8172-8376	8228	1-8227 and 8229-8376
8115	1-8114 and 8116-8376	8172	1-8171 and 8173-8376	8229	1-8228 and 8230-8376
8116	1-8115 and 8117-8376	8173	1-8172 and 8174-8376	8230	1-8229 and 8231-8376
8117	1-8116 and 8118-8376	8174	1-8173 and 8175-8376	8231	1-8230 and 8232-8376
8118	1-8117 and 8119-8376	8175	1-8174 and 8176-8376	8232	1-8231 and 8233-8376
8119	1-8118 and 8120-8376	8176	1-8175 and 8177-8376	8233	1-8232 and 8234-8376
8120	1-8119 and 8121-8376	8177	1-8176 and 8178-8376	8234	1-8233 and 8235-8376
8121	1-8120 and 8122-8376	8178	1-8177 and 8179-8376	8235	1-8234 and 8236-8376
8122	1-8121 and 8123-8376	8179	1-8178 and 8180-8376	8236	1-8235 and 8237-8376
8123	1-8122 and 8124-8376	8180	1-8179 and 8181-8376	8237	1-8236 and 8238-8376
8124	1-8123 and 8125-8376	8181	1-8180 and 8182-8376	8238	1-8237 and 8239-8376
8125	1-8124 and 8126-8376	8182	1-8181 and 8183-8376	8239	1-8238 and 8240-8376
8126	1-8125 and 8127-8376	8183	1-8182 and 8184-8376	8240	1-8239 and 8241-8376
8127	1-8126 and 8128-8376	8184	1-8183 and 8185-8376	8241	1-8240 and 8242-8376
8128	1-8127 and 8129-8376	8185	1-8184 and 8186-8376	8242	1-8241 and 8243-8376
8129	1-8128 and 8130-8376	8186	1-8185 and 8187-8376	8243	1-8242 and 8244-8376
8130	1-8129 and 8131-8376	8187	1-8186 and 8188-8376	8244	1-8243 and 8245-8376
8131	1-8130 and 8132-8376	8188	1-8187 and 8189-8376	8245	1-8244 and 8246-8376
8132	1-8131 and 8133-8376	8189	1-8188 and 8190-8376	8246	1-8245 and 8247-8376
8133	1-8132 and 8134-8376	8190	1-8189 and 8191-8376	8247	1-8246 and 8248-8376
8134	1-8133 and 8135-8376	8191	1-8190 and 8192-8376	8248	1-8247 and 8249-8376
8135	1-8134 and 8136-8376	8192	1-8191 and 8193-8376	8249	1-8248 and 8250-8376
8136	1-8135 and 8137-8376	8193	1-8192 and 8194-8376	8250	1-8249 and 8251-8376
8137	1-8136 and 8138-8376	8194	1-8193 and 8195-8376	8251	1-8250 and 8252-8376
8138	1-8137 and 8139-8376	8195	1-8194 and 8196-8376	8252	1-8251 and 8253-8376
8139	1-8138 and 8140-8376	8196	1-8195 and 8197-8376	8253	1-8252 and 8254-8376
8140	1-8139 and 8141-8376	8197	1-8196 and 8198-8376	8254	1-8253 and 8255-8376
8141	1-8140 and 8142-8376	8198	1-8197 and 8199-8376	8255	1-8254 and 8256-8376
8142	1-8141 and 8143-8376	8199	1-8198 and 8200-8376	8256	1-8255 and 8257-8376
8143	1-8142 and 8144-8376	8200	1-8199 and 8201-8376	8257	1-8256 and 8258-8376
8144	1-8143 and 8145-8376	8201	1-8200 and 8202-8376	8258	1-8257 and 8259-8376
8145	1-8144 and 8146-8376	8202	1-8201 and 8203-8376	8259	1-8258 and 8260-8376
8146	1-8145 and 8147-8376	8203	1-8202 and 8204-8376	8260	1-8259 and 8261-8376
8147	1-8146 and 8148-8376	8204	1-8203 and 8205-8376	8261	1-8260 and 8262-8376
8148	1-8147 and 8149-8376	8205	1-8204 and 8206-8376	8262	1-8261 and 8263-8376
8149	1-8148 and 8150-8376	8206	1-8205 and 8207-8376	8263	1-8262 and 8264-8376
8150	1-8149 and 8151-8376	8207	1-8206 and 8208-8376	8264	1-8263 and 8265-8376
8151	1-8150 and 8152-8376	8208	1-8207 and 8209-8376	8265	1-8264 and 8266-8376
8152	1-8151 and 8153-8376	8209	1-8208 and 8210-8376	8266	1-8265 and 8267-8376
8153	1-8152 and 8154-8376	8210	1-8209 and 8211-8376	8267	1-8266 and 8268-8376
8154	1-8153 and 8155-8376	8211	1-8210 and 8212-8376	8268	1-8267 and 8269-8376
8155	1-8154 and 8156-8376	8212	1-8211 and 8213-8376	8269	1-8268 and 8270-8376
8156	1-8155 and 8157-8376	8213	1-8212 and 8214-8376	8270	1-8269 and 8271-8376
8157	1-8156 and 8158-8376	8214	1-8213 and 8215-8376	8271	1-8270 and 8272-8376
8158	1-8157 and 8159-8376	8215	1-8214 and 8216-8376	8272	1-8271 and 8273-8376
8159	1-8158 and 8160-8376	8216	1-8215 and 8217-8376	8273	1-8272 and 8274-8376
8160	1-8159 and 8161-8376	8217	1-8216 and 8218-8376	8274	1-8273 and 8275-8376
8161	1-8160 and 8162-8376	8218	1-8217 and 8219-8376	8275	1-8274 and 8276-8376
8162	1-8161 and 8163-8376	8219	1-8218 and 8220-8376	8276	1-8275 and 8277-8376
8163	1-8162 and 8164-8376	8220	1-8219 and 8221-8376	8277	1-8276 and 8278-8376
8164	1-8163 and 8165-8376	8221	1-8220 and 8222-8376	8278	1-8277 and 8279-8376
8165	1-8164 and 8166-8376	8222	1-8221 and 8223-8376	8279	1-8278 and 8280-8376

8280	1-8279 and 8281-8376	8313	1-8312 and 8314-8376	8346	1-8345 and 8347-8376
8281	1-8280 and 8282-8376	8314	1-8313 and 8315-8376	8347	1-8346 and 8348-8376
8282	1-8281 and 8283-8376	8315	1-8314 and 8316-8376	8348	1-8347 and 8349-8376
8283	1-8282 and 8284-8376	8316	1-8315 and 8317-8376	8349	1-8348 and 8350-8376
8284	1-8283 and 8285-8376	8317	1-8316 and 8318-8376	8350	1-8349 and 8351-8376
8285	1-8284 and 8286-8376	8318	1-8317 and 8319-8376	8351	1-8350 and 8352-8376
8286	1-8285 and 8287-8376	8319	1-8318 and 8320-8376	8352	1-8351 and 8353-8376
8287	1-8286 and 8288-8376	8320	1-8319 and 8321-8376	8353	1-8352 and 8354-8376
8288	1-8287 and 8289-8376	8321	1-8320 and 8322-8376	8354	1-8353 and 8355-8376
8289	1-8288 and 8290-8376	8322	1-8321 and 8323-8376	8355	1-8354 and 8356-8376
8290	1-8289 and 8291-8376	8323	1-8322 and 8324-8376	8356	1-8355 and 8357-8376
8291	1-8290 and 8292-8376	8324	1-8323 and 8325-8376	8357	1-8356 and 8358-8376
8292	1-8291 and 8293-8376	8325	1-8324 and 8326-8376	8358	1-8357 and 8359-8376
8293	1-8292 and 8294-8376	8326	1-8325 and 8327-8376	8359	1-8358 and 8360-8376
8294	1-8293 and 8295-8376	8327	1-8326 and 8328-8376	8360	1-8359 and 8361-8376
8295	1-8294 and 8296-8376	8328	1-8327 and 8329-8376	8361	1-8360 and 8362-8376
8296	1-8295 and 8297-8376	8329	1-8328 and 8330-8376	8362	1-8361 and 8363-8376
8297	1-8296 and 8298-8376	8330	1-8329 and 8331-8376	8363	1-8362 and 8364-8376
8298	1-8297 and 8299-8376	8331	1-8330 and 8332-8376	8364	1-8363 and 8365-8376
8299	1-8298 and 8300-8376	8332	1-8331 and 8333-8376	8365	1-8364 and 8366-8376
8300	1-8299 and 8301-8376	8333	1-8332 and 8334-8376	8366	1-8365 and 8367-8376
8301	1-8300 and 8302-8376	8334	1-8333 and 8335-8376	8367	1-8366 and 8368-8376
8302	1-8301 and 8303-8376	8335	1-8334 and 8336-8376	8368	1-8367 and 8369-8376
8303	1-8302 and 8304-8376	8336	1-8335 and 8337-8376	8369	1-8368 and 8370-8376
8304	1-8303 and 8305-8376	8337	1-8336 and 8338-8376	8370	1-8369 and 8371-8376
8305	1-8304 and 8306-8376	8338	1-8337 and 8339-8376	8371	1-8370 and 8372-8376
8306	1-8305 and 8307-8376	8339	1-8338 and 8340-8376	8372	1-8371 and 8373-8376
8307	1-8306 and 8308-8376	8340	1-8339 and 8341-8376	8373	1-8372 and 8374-8376
8308	1-8307 and 8309-8376	8341	1-8340 and 8342-8376	8374	1-8373 and 8375-8376
8309	1-8308 and 8310-8376	8342	1-8341 and 8343-8376	8375	1-8374 and 8376
8310	1-8309 and 8311-8376	8343	1-8342 and 8344-8376	8376	1-8375
8311	1-8310 and 8312-8376	8344	1-8343 and 8345-8376		
8312	1-8311 and 8313-8376	8345	1-8344 and 8346-8376		

Thus the invention includes each of the 35074500 possible pairs of SEQ IDs 1-8376 (1&2, 1&3, 1&4, 1&5 ... 1&8375, 1&8376, 2&3, 2&4, 2&5 ... 2&8375, 2&8376, 3&4 ... 5 1000&1001, 1000&1002 ... 1000&8376 ... 8374&8375, 8374&8376, 8375&8376) although, for reasons of space, these are not listed in full here.

- Details as to how the molecules which make up the SEQ IDs 1-4056 can be produced and used can be found from the relevant international applications and these details need not be repeated here. Similar principles apply to SEQ IDs 4057-8376.
- 10 SEQ IDs 1-8376 in the compositions of the invention may be supplemented or substituted with molecules comprising sequences homologous (*ie.* having sequence identity) to SEQ IDs 1-8376. Depending on the particular sequence, the degree of identity is preferably greater than 50% (*eg.* 65%, 80%, 90%, or more), and include mutants and allelic variants. Sequence identity between the proteins is preferably determined by the Smith-Waterman homology search

algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1.

SEQ IDs 1-8376 in the compositions of the invention may be supplemented or substituted with molecules comprising fragments of SEQ IDs 1-8376. Such fragments should comprise at least *n* consecutive monomers from the molecules and, depending on the particular sequence, *n* is either (i) 7 or more for protein molecules (eg. 8, 10, 12, 14, 16, 18, 20 or more), preferably such that the fragment comprises an epitope from the sequence, or (ii) 10 or more for nucleic acid molecules (eg 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

Where the composition includes a protein that exists in different nascent and mature forms, the mature form of the protein is preferably used. For example, the mature form of the NspA protein (SEQ IDs 4008-4033; WO96/29412; Figure 29) lacking the signal peptide may be used.

In the case of protein molecules, SEQ IDs 1-8376 in the compositions of the invention may be supplemented or substituted with an antibody that binds to the protein. This antibody may be monoclonal or polyclonal.

15 In the case of nucleic acid molecules, SEQ IDs 1-8376 in the compositions of the invention may be supplemented or substituted with nucleic acid which can hybridise to the Neisseria nucleic acid, preferably under "high stringency" conditions (eg. 65°C in a 0.1xSSC, 0.5% SDS solution).

It will be appreciated that any nucleic acid in the compositions can take various forms (eg. 20 single stranded, double stranded, vectors, probes etc.). In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) etc.

25 In certain embodiments, the composition comprises molecules from different *Neisseria* species, such as one or more *N.meningitidis* molecule and one or more *N.gonorrhoeae* molecule. In some embodiments, the composition may comprise molecules from different serogroups and/or strains of the same species, such as strains A and B of *N.meningitidis*. Further embodiments comprise mixtures of one or more *N.meningitidis* molecules from different strains and also one or more *N.gonorrhoeae* molecules.

Many proteins are relatively conserved between different species, serogroups and strains of 30 *N.meningitidis* and *N.gonorrhoeae* (eg. SEQ IDs 52, 54, 58). PCT/IB00/00642 includes a more

detailed experimental analysis of conserved regions in these proteins. To ensure maximum cross-strain recognition and reactivity, regions of proteins that are conserved between different Neisserial species, serogroups and strains can be used in the compositions of the present invention. The invention therefore provides proteins which comprise stretches of amino acid sequence that are shared across the majority of *Neisseria*, particularly *N.meningitidis* and *N.gonorrhoeae*. Preferably, therefore, the composition comprises a protein comprising a fragment of a Neisserial protein (preferably a protein from SEQ IDs 1-8376, or more preferably SEQ IDs 1-4002), wherein said fragment consists of n consecutive conserved amino acids. Depending on the particular protein, n is 7 or more (eg. 8, 10, 12, 14, 16, 18, 20 or more). The fragment preferably comprises an antigenic or immunogenic region of the Neisserial protein. A "conserved" amino acid is one that is present in a particular Neisserial protein in at least $x\%$ of *Neisseria* (or, preferably, in at least $x\%$ of combined *N.meningitidis* and *N.gonorrhoeae* strains). The value of x may be 50% or more eg. 66%, 75%, 80%, 90%, 95% or even 100% (ie. the amino acid is found in the protein in question in all *Neisseria*). In order to determine whether an amino acid is "conserved" in a particular Neisserial protein, it is necessary to compare that amino acid residue in the sequences of the protein in question from a plurality of different *Neisseria* (a "reference population"). Suitable definitions of "reference populations" can be found in PCT/IB00/00642. Amino acid sequences of different *Neissieriae* can easily be compared using computers. This will typically involve the alignment of a number of sequences using an algorithm such as CLUSTAL [Thompson *et al.* (1994) *Nucleic Acids Res* 22:4673-4680; *Trends Biochem Sci* (1998) 23:403-405] or, preferably, PILEUP [part of the GCG Wisconsin package, preferably version 9.0]. Conserved amino acids are readily apparent in a multiple sequence alignment – at the amino acid position in question a majority of the aligned sequences will contain a particular amino acid. Conserved amino acids can be made more visually apparent by using a program such as BOXSHADE [available, for instance, at the NIH on-line], PRETTYBOX [GCG Wisconsin, version 10] or JALVIEW [available on-line at EBI].

Specific compositions according to the invention therefore include those comprising:

- two or more biological molecules selected from SEQ IDs 1-4002;
- one or more biological molecules selected from SEQ IDs 1-4002 combined with one or 30 more biological molecules selected from SEQ IDs 4003-8376;
- one or more biological molecules selected from SEQ IDs 1-4002 combined with the NspA protein (as disclosed in WO96/29412: see also Figure 29 herein), preferably in mature form;

- one or more biological molecules selected from SEQ IDs 1-8376 (preferably SEQ IDs 1-4002) combined with transferrin binding protein A (TbpA) and/or B (TbpB), such as the TbpA and TbpB disclosed in WO00/25811 (or immunogenic fragments thereof).
- one or more fragments of proteins selected from SEQ IDs 1-4002, with the fragment preferably comprising a stretch of conserved amino acids;
- a combination of different proteins, wherein the combination as a whole includes one or more proteins that is recognised by each strain in a reference population, although each individual protein in the combination may not itself be recognised by each strain in the reference population *ie.* each member of a reference population recognises at least one protein in the combination.

The invention also provides the compositions of the invention for use as medicaments (*eg.* as immunogenic compositions or vaccines) or as diagnostic reagents. It also provides the use of the compositions in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to the invention.

The invention further provides a process for producing a composition according to the invention, comprising the step of bringing one or more of SEQ IDs 1-8376 into combination with one or more other of SEQ IDs 1-8376.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows SDS-PAGE results for the expression of ORFs 6, 7, 13, 65-1, 72, 73-1, 105-1, 137-1, 143-1 and 147-1. The left-hand lanes show molecular weight markers (set M1).

Figure 2 shows (A) SDS-PAGE results for ORF9 (B) the position of the *N.meningitidis* immunoreactive band in a Western blot against a *N.meningitidis* outer membrane vesicle preparation (C) FACS analysis.

Figure 3 shows SDS-PAGE results for the expression of ORFs 2-1, 5-1, 22-1, 132-1 and 4. The left-hand lanes show molecular weight markers (set M1).

Figures 4 to 28 show a hydrophilicity plot (upper), antigenic index (middle), and AMPHI regions (lower) for ORFs 2, 5, 6, 7, 9, 13a, 15, 22, 23, 27, 28, 32, 65, 72, 73, 76, 79, 89, 105, 106-1, 132, 137, 138, 143 and 147.

Figure 29 shows sequence variability of NspA from various strains of meningococcus B. These 5 sequences may be used as alternatives to the NspA of WO 96/29412 (SEQ IDs 4008-4033).

Figure 30 shows the binding of polyclonal anti-rNspA by indirect fluorescence flow cytometry to encapsulated and non-encapsulated menB strains. **Figure 31** shows similar data for encapsulated strains 8047, CU385 & M986 (31A) and non-encapsulated strains BZ232, MC58, NG3/88 & NGB165 (31B).

10 **Figure 32** shows a model of NspA secondary structure.

Figure 33 shows FACS analysis of non-encapsulated strain M7 (37A) and ethanol-treated (to disrupt the capsule) strains 2996, N44776, MC58, 1000, BZ232, BZ133, NG6/88, BZ198, NG3/88, 297-0, BZ147 and BZ169 (37B) using a tetravalent mixture.

Figure 34 shows FACS analysis of M7 strain using a pentavalent mixture at 1:400 (34A), 1:200 15 (34B) & 1:100 (34C) dilutions.

EXAMPLES

Example 1 – Expression and purification experiments

ORFs 6, 7, 13, 65-1, 72, 73-1, 105-1, 137-1, 143-1 and 147-1 disclosed in WO99/24578 were expressed in *E.coli* and purified, as set out in the following table:

ORF	His-fusion	GST-fusion	Purification	MW (kDa)
6		+	GST-fusion	23
7	+	+	GST-fusion	28
13		+	GST-fusion	10
65-1		+	GST-fusion	32
72	+		His-fusion	13.5
73-1		+	GST-fusion	13
105-1	+	+	His-fusion	32
137-1		+	GST-fusion	31
143-1	+	+	His-fusion	23.5
147-1	-	+	His-fusion	32

NOTE: ORF73-1 was expressed as a fragment (amino acids 41-161)

The protocols used to express these ten ORFs were essentially the same as those described in WO99/24578, using pGEX and pET vectors. Examples of the PCR primers used to amplify the ORFs are in the following table:

ORF	Primer	Sequence	Restriction sites
ORF65-1	Forward	CGCGGATCCCATATG-TTTATGAACAAATTTC	BamHI-NdeI Xhol
	Reverse	CCCG <u>CTCGAG</u> -TTTGCTTCGATAGAACGG	
ORF73-1	Forward	CGCGGATCCCATATG-GCCGGCGTGCTGATG	BamHI-NdeI Xhol
	Reverse	CCCG <u>CTCGAG</u> -TTCATCTTTCATGTTCG	
ORF105-1	Forward	CGCGGATCCCATATG-CCGACCGTCCGTT	BamHI-NdeI Xhol
	Reverse	CCCG <u>CTCGAG</u> -TAAACGAATGCCGTCCAG	
ORF143-1	Forward	CGCGGATCCCATATG-GAATCAACACTTCAC	BamHI-NdeI Xhol
	Reverse	CCCG <u>CTCGAG</u> -CACGCGGTTGCTGTAAC	
ORF147-1	Forward	CCG <u>GAATTCTACATATG</u> -TTTCAGAAACATTGCAG	EcoRI-NdeI Xhol
	Reverse	CCCG <u>CTCGAG</u> -TTTGTCCCCAAGACAGAG	

5 SDS-PAGE results for the ten expressed ORFs are shown in Figure 1.

The ORF7-His fusion was used to immunise mice. The sera were used in an ELISA assay, essentially as described in WO99/24578, and gave positive results.

The following proteins were also expressed and purified (results not shown):

ORF	His-fusion expression	GST-fusion expression	Expressed as a fragment	Fragment amino acid positions
orf1 (s)	-	-	-	43-1087
orf1 (β)	n.d.	-	-	1051-1457
orf35-1	-	-	-	
orf41	n.d.	n.d.	-	25-619
orf46	-	-	-	25-433
orf61	n.d.	n.d.	-	1-575

orf83-1	-	-	-	15-313
orf100-1	n.d.	-	-	21-376
orf114-1	n.d.	-	-	1-1423
orf124-1	n.d.	n.d.	-	
orf131-1	n.d.	-	-	22-135

The following PCR primers were used to amplify these ORFs:

ORF	Primer	Sequence	Restriction sites
ORF1 (α)	Forward	CGCGGATCCGCTAGC-GGACACACTTATTCGG	BamHI-NheI
	Reverse	CCCG <u>CTCGAG</u> -CAGCGCGTCAAGGCTT	XhoI
ORF1 (β)	Forward	CGCGGATCCGCTAGC-GAGTTCCGCCTGCATAA	BamHI-NheI
	Reverse	CCCG <u>CTCGAG</u> -CCAGCGGTAGCCTAATT	XhoI
ORF35-1	Forward	CGCGGATCCGCTAGC-AGGGGCGACGACGTG	BamHI-NheI
	Reverse	CCCG <u>CTCGAG</u> -AAACAGCCATTGAGCGA	XhoI
ORF41	Forward	CGCGGATCCCATATG-TATTGAAACAGCTCCAAG	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -TTCTGGGTGAATGTTA	XhoI
ORF46	Forward	CGCGGATCCCATATG-TCAGATTGGCAAACGATC	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -CGTATCATATTCACGTGC	XhoI
ORF61	Forward	CGCGGATCCGCTAGC-ACGGTTTGAAAGCTTCGC	BamHI-NheI
	Reverse	CCCG <u>CTCGAG</u> -AATGACGAGGTTGTCCG	XhoI
ORF83-1	Forward	CGCGGATCCCATATG-TGCGGCACACTGACCG	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -GCCGCCTTGCAGC	XhoI
ORF100-1	Forward	CGCGGATCCCATATG-TCGGGCATTTACACCGG	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -TTCGTCGAAAACCTTTGC	XhoI
ORF114-1	Forward	CGCGGATCCCATATG-AATAAAGGTTACATCGCAT	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -AATCGCTGCACCGGCT	XhoI
ORF124-1	Forward	CGCGGATCCCATATG-ACTGCCTTTCGACA	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -GCGTGAAGCGTCAGGA	XhoI

ORF131-1	Forward Reverse	<u>CGCGGATCCC</u> <u>CATATG</u> -TGCCGGCTGGCGGG <u>CCCGCTCGAG</u> -CCAGCGGACGCCGTTC	BamHI-NdeI XbaI
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Each of these ORFs can be combined with one or more of SEQ IDs 1-8376.

Example 2 – ORF9 expression and purification

ORF9 disclosed in WO99/24578 was cloned in the pET vector and expressed in *E.coli*. The purified ORF-His fusion protein was analysed by SDS-PAGE, as shown in Figure 2A. Mice 5 were immunised with the purified ORF9-His and sera were used for Western blot analysis (Figure 2B). FACS analysis (Figure 2C), and ELISA assay. The protocols used were essentially the same as those set out in WO99/24578.

The results confirm that ORF9 is a surface-exposed protein. ORF9 is suitable for combining with one or more other of SEQ IDs 1-8376.

10 Example 3 – Further expression experiments

Further expression and purification experiments were carried out in *E.coli* for ORFs 2-1, 5-1, 22-1 and 132-1 disclosed in WO99/24578, as set out in the following table:

ORF	His-fusion	GST-fusion	Purification	MW (kDa)
2-1		+	GST-fusion	26
5-1	-	+	GST-fusion	33
22-1	+	+	His-fusion	49
132-1	-	+	GST-fusion	48

The protocols used to express these four ORFs were essentially the same as those described in 15 WO99/24578, using pGEX and pET vectors. Examples of the PCR primers used to amplify the ORFs are in the following table:

ORF	Primer	Sequence	Restriction sites
ORF5-1	Forward	<u>CGCGGATCC</u> <u>GCTAGC</u> -GACGGCGCACAAACCGA	BamHI-NheI

	Reverse	CCCG <u>CTCGAG</u> -TGTTGGCGGATGGGG	Xhol
ORF22-1	Forward Reverse	CGCG <u>GGATCCC</u> CATATG-ATTAAAATCAAAAAAGGTC CCCG <u>CTCGAG</u> -GCCTCCTTCTCAATGG	BamHI-NdeI Xhol
ORF132-1	Forward Reverse	CGCG <u>GGATCCC</u> CATATG-AAAGAAGCAGGGTTGAA CCCG <u>CTCGAG</u> -TCTCAAAGCTTCCAGCAG	BamHI-NdeI Xhol

SDS-PAGE results for the four expressed ORFs are shown in Figure 3.

Each of these ORFs can be combined with one or more of SEQ IDs 1-8376.

Example 4 – ORF4 lipoprotein expression and purification

ORF4 is disclosed in WO99/24578 as containing a lipopeptide signal sequence (LPSS). The full

5 length ORF was amplified using the following PCR primers:

orf4-L for CGCGGATCCCATATGAAAACCTTCTTCAAAACC

orf4-L rev CCCGCTCGAGTTATTGGCTGCGCCTTC

The amplified DNA fragment was cloned into the vector pET21b+ for expression as a C-terminus His-tagged fusion. A log phase culture of *E.coli* containing pET21b+orf4-LPSS was

10 induced with 1.0 mM IPTG for 3 h at 30°C. collected by centrifugation for 10 min at 8000g, and resuspended in PBS. The suspension was sonicated on ice and Triton X-114 added to a final concentration of 0.6% (v/v). The material was incubated on ice for 20 minutes then warmed to 37°C until phase separation occurred (indicated by a high degree of cloudiness).

After centrifugation for 10 min at 10,000g at 20°C the upper aqueous phase was discarded, and 15 the lower detergent phase collected without disrupting the bacterial pellet. To the detergent phase was added 13 volumes of 20 mM Histidine, 2 mM EDTA, 30 mM NaCl (pH 5.8). This was centrifuged for 10 min at 4°C and the supernatant combined batchwise at 4°C for 30 min, with Q Sepharose Fast Flow resin (Pharmacia). The mixture was centrifuged, the supernatant retained and the resin washed with 20mM Histidine, 2mM EDTA, 30 mM NaCl (pH 5.8),

20 Triton X-100 0.3% (v/v) and eluted with 1M NaCl in the same buffer. The majority of Orf4 lipoprotein was found in the supernatant obtained after binding. Final purification was accomplished by chromatography on Hi-Trap™ Q (Pharmacia). The binding-supernatant was adjusted to pH 7.0 by the addition of 0.1M HCl and applied to a Hi-Trap™ Q column

equilibrated with 50mM Tris-HCl (pH 7.0), 2mM EDTA, 0.3% Triton X-100, 10mM NaCl. The column was washed with 5.0 ml of the equilibration buffer and a NaCl gradient from 10mM to 1M was applied. Two electrophoretically distinct forms of the protein eluted. One in the wash and the other in the NaCl gradient between 150mM and 300mM NaCl. The protein obtained in the wash was used for immunization of mice. This form of the protein probably represents the fully processed, lipidated molecule.

The 31kDa purified lipoprotein can be seen in Figure 3. ORF4 is suitable for combining with one or more other of SEQ IDs 1-8376.

Example 5 – Computer prediction

Computer analysis of ORFs 2, 5, 6a, 7, 9, 13a, 15, 22, 23, 27, 28, 32, 65, 72, 73, 76, 79, 89, 105, 106-1, 132, 137, 138, 143 and 147 (as disclosed in WO99/24578) was performed. Figures 4 to 28 show, for each of these ORFs, a hydrophilicity plot (upper), an antigenic index plot (middle), and an AMPHI analysis (lower). The AMPHI program has been used to predict T-cell epitopes [Gao *et al.* (1989) *J. Immunol.* 143:3007; Roberts *et al.* (1996) *AIDS Res Hum Retrovir* 12:593; Quakyi *et al.* (1992) *Scand J Immunol* suppl.11:9] and is available in the Protean package of DNASTAR, Inc. (1228 South Park Street, Madison, Wisconsin 53715 USA).

Each of these ORFs can be combined with one or more other of SEQ IDs 1-8376.

Example 6 – Tetravalent mixture

A mixture of proteins 919 (WO99/57280), 225 (WO99/57280), ORF4 (WO99/24578, example 26) and ORF40 (WO99/36544, example 1) was produced and assessed by ELISA and FACS. The ELISA titres against 13 test strains were as follows:

Strain	M7 conF	2996	MC58	BZ133	BZ232	H44/76	1000
Titre	38176	7892	17216	41488	17945	83990	6575
Strain	BZ198	NG6/88	BZ169	BZ147	NG3/88	297-0	
Titre	3329	59275	-	-	12877	25640	

The FACS results are shown in Figure 33. It is evident that the tetravalent mixture gives excellent results, regardless of the particular menB strain used. In addition, antisera raised against the mixture in strain 2996 is bactericidal at up to 1:2048 dilution.

Example 7 – Pentavalent mixture

A mixture of proteins ORF4-L (the lipidated protein – see example 4 above), ORF37 (WO99/24578, example 1), ORF40 (WO99/36544, example 1), 502 (WO99/57280, pages 687-690) and 8 (WO99/57280, pages 165-167) was produced. The ELISA titres against 13 test strains were as follows:

Strain	M7 conF	2996	MC58	BZ133	BZ232	H44/76	1000
Titre	-	-	25428	-	58300	>109350	>109350
Strain	BZ198	NG6/88	BZ169	BZ147	NG3/88	297-0	
Titre	10999	109532	42888	28324	104212	33996	

The FACS results are shown in Figure 34. It is evident that the pentavalent mixture gives excellent results, regardless of the particular menB strain used. In addition, antisera raised against the mixture in strain 2996 is bacteriostatic.

10 Example 8 – trivalent mixture

Proteins ORF1 (e.g. example 77 of WO99/24578; see also WO99/55873), '287' (e.g. Figure 21 of WO99/57280; also SEQ IDs 3103-3108 therein) and '919' (e.g. WO99/57280 Figure 23 and SEQ IDs 3069-3074 therein) were combined and adjuvanted with Al(OH)₃. The proteins were from the 2996 strain of MenB.

15 This mixture was also combined with a MenC polysaccharide conjugate antigen [e.g. Costantino *et al.* (1992) *Vaccine* 10:691-698]. OMVs were used as controls.

The mixture was used in a bactericidal assay against the homologous strain and also heterologous MenB strains. Titres were as follows:

	2996	BZ133	BZ232	1000	MC58	NGH38
Trivalent	2048	2048	4	<4	64	4
+ MenC	2048	>32000	4	128	1024	128
Control	32765	4096	8192	16384	16384	8192

Example 9 – Proteins 287, 919 and 953

Proteins 287, 919 and 953 are disclosed in WO99/57280. These proteins from *N.meningitidis* serogroup B strain 2996 were expressed and tested in a bactericidal assay against strain 2996, alone and in combinations. OMVs from 2996 were used as a positive control.

Antigen	287	919	953	Control
Titre	8192	2048	128	65536
Combination	287+919	287+953	919+953	287+919+953
Titre	32000	8192	8192	8192

5

Figure 35 shows FACS data for the individual antigens and for the four combinations.

It is evident that the antigen mixtures are more effective than the antigens in isolation and, in particular, that combinations of 919+953 give surprisingly good results.

The individual antigens from 2996 and combinations were also tested against different 10 serogroup A, B & C strains (*i.e.* heterologous challenge). Bactericidal titres were as follows:

Antigen	Serogroup B (MenB) strains					MenA	MenC
	2996	BZ133	BZ232	MC58	NGH38	F6124	C11
287	8192	>4096	256	1024	2048	1024	2048
919	2048	-	1024	-	-	-	-
953	128	-	-	-	-	-	-
287+919	32000	>4096	512	512	1024	512	>2048
287+953	8192	>4096	1024	512	2048	2048	>2048
919+953	8192	-	8192	-	-	-	-
Trivalent	8192	>2048	256	-	1024	>2048	>2048
Control	65536	-	8192	2048	-	2048	32768

It is apparent that the antigen mixtures are useful in conferring cross-strain activity.

In a second set of experiments, titres for the individual antigens were as follows:

Antigen	Serogroup B (MenB) strains					MenA	MenC
	2996	BZ133	BZ232	MC58	NGH38	F6124	C11
287	16000	2048	16	512	>2048	64	1024
919	16000	-	2048	-	-	-	-
953	2048	-	16	-	-	-	-

The three proteins used in this example were expressed and used in the following forms:

- (1) Protein 287 was expressed in *E.coli* as a GST fusion;
- (2) Protein 919 was expressed in *E.coli* without its leader peptide, without its mature N-terminal cysteine, and without any fusion partners ("919-untag"); and
- (3) Protein 953 was expressed using a histidine tag.
- 5 Three immunisations were administered with Freund's adjuvants – the first included CFA, and the final two included IFA.

Example 10 – further polyvalent combinations

Further combinations of antigens were tested in CD1 mice:

Antigens *	Adjuvant	FACS	ELISA	Bactericidal activity
919-his+Orf4-his+ 225-his+Orf40-his	Freund	+++	+	8192
Orf4-L+Orf37-GST+Orf40-his+502-his+8-his	Freund	+++	+	Bacteriostatic
919-untag+791-his+792-his	Freund	+++	+	4096
919-untag+287-GST+953-his	Freund	+++	+	8192
919-untag+287-GST	Freund	+++	+	32000
287-GST+953-his	Freund	+++	+	8192
919-untag+953-his	Freund	+++	+	8192
919-untag+Orf1-his+287-GST	Al(OH) ₃	+++	+	2048
919-untag+Orf1-his+287-GST+MenC glycoconj.	Al(OH) ₃	+++	+	2048
Orf-46.1-his+ 287-GST	Al(OH) ₃	n.d.	+	128

* : "his" indicates expression and immunisation with a histidine-tagged protein;
 10 "ORF4-L" is the lipidated form of ORF4;
 "GST" indicates expression and immunisation with a GST fusion protein;
 "919-untag" is as defined in Example 9;
 "MenC glycoconj" is the MenC glycoconjugate described in Example 8.

Further combinations of antigens were tested in guinea pigs:

Antigens	Adjuvant	FACS	ELISA	Bactericidal activity
919-his+287-GST+953-his+Orf46.1-his	Freund	+	+	4096
919-untage + 287-GST + 953-his	Freund	+	+	4096
287-GST + 953-his	Al(OH) ₃	n.d.	+	256

Evidently the combinations give excellent immunological results.

Example 11 – NspA combinations

NspA protein is disclosed in WO96/29412, and is represented herein as SEQ IDs 4008-4033.

The academic literature disclosure of this protein [Martin *et al.* (1997) *J. Exp. Med.* 185 1173-5 1183] reported the protein to be highly conserved between *Neisseria* strains (99% cross-reactivity of anti-NspA antibodies with 250 meningococcal A, B & C strains) and also efficient protection against deadly challenge with live bacteria. There have also been reports that NspA adsorbed on alum elicits serum meningococcal bactericidal antibody responses in rabbits and monkeys [Martin *et al.* (1998) *Abstracts of 11th International pathogenic Neisseria conference*, 10 page 198]. On the basis of these data, rNspA (recombinant NspA) is being developed as a vaccine for the prevention of meningococcal disease caused by all serogroups.

Despite sequence conservation, however, it has surprisingly been discovered that rNspA cell surface epitopes are detected on only 65% of the serogroup B strains tested below, and susceptibility to anti-NspA bactericidal activity is also less than that reported by Martin *et al.* 15 These results contrast with Martin *et al.*, and suggest that a rNspA-based meningococcal B vaccines will need to be supplemented with additional antigens in order to be effective.

The *N.meningitidis* strains tested in this example were isolated from patients residing in different countries over a period of more than 30 years (see table on page 72). These strains were selected to be representative of widely divergent 'clonal' groups, as defined by multilocus 20 isoenzyme typing [Seiler *et al.* (1996) *Mol. Microbiol.* 19:841-856] and/or multilocus sequence typing [Maiden *et al.* (1998) *PNAS USA* 95:3140-45]. Strain M7, which is derived from strain NMB, contains a transposon insertion that blocks capsular polysaccharide biosynthesis [Stephens *et al.* (1991) *Infect. Immun.* 59:4097-4102], but all the other strains are encapsulated.

Based on the nucleotide sequence in Martin *et al.* (1997), PCR primers were designed and the 25 NspA gene from strain 8047 was amplified. The sequence, including the promoter region, was cloned into pSK+ plasmid (rNspA). A plasmid pTrc.NspA.1 encoding a protein in which a portion of the signal sequence has been replaced with a poly-histidine tag was also used. Both plasmids were expressed in *E.coli* strain BL21(DE3) and the proteins were purified. In *E.coli*, rNspA is secreted, rather than being associated with the outer membrane. The protein was 30 partially purified from the culture medium by precipitation with 55% w/v ammonium sulphate, and had an apparent MW of 18.6kDa. confirmed by Western Blot.

The two forms of NspA (rNspA and denature His-tag NspA) were injected into 6-week old female CD-1 mice to raise antisera. The ability of these to bind to the surface of *N.meningitidis* strain B was determined using flow cytometric detection of indirect fluorescence assay [Granoff *et al.* (1998) *J.Immunol.* 160:5028-36]. The results for strains NMB and M7 (an acapsulated 5 mutant of NMB) are shown in Figure 30. As expected, anti-group B polysaccharide mAb SEAM-3 [Granoff *et al.*] only binds to the encapsulated strain, whereas the positive anti-P1.2 (PorA) control mAb binds to both strains. The antisera raised against rNspA is able to bind both strains. Antisera against the His-tag NspA gave negative results, however. These antisera were also negative for strains 8047, CU385 and M986 (Figure 31A), but by Western Blot these 10 antisera gave positive results.

These data suggest that antibodies prepared using His-tag NspA recognise epitopes that are present in denatured NspA, but not native NspA as found on the cell-surface *in vivo*. In contrast, antibodies prepared against rNspA seem to recognise conformational NspA epitopes.

The flow cytometric assay was applied to the strains shown in the table on page 72. Figure 31A 15 shows that murine antibodies raised against rNspA bind to the surface of strain 8047 (the strain from which the *nspA* gene was cloned) and strain CU385, but not to M986. Figure 31B shows similar negative results for strains BZ232, MC58, NG3/88 and NGP165. In all of these negative cases, however, the anticapsular mAb control was positive.

The table on page 72 summarises the flow cytometry results. Although NspA is reported to be 20 accessible at the surface of all intact *N.meningitidis* strains tested [Martin *et al.* (1997) *J. Exp. Med.* 185: 1173-1183; Plante *et al.* (1999) *Infect. Immun.* 67:2855-61], only 11 of the 17 test strains (65%) reacted with the anti-rNspA sera. There was no apparent relationship between cell-surface expression in a given strain and classification (by serotype, subtype, or electrophoretic type), or with year or country of isolation.

25 In an attempt to explain the differences in reactivity with the anti-rNspA sera, the *nspA* genes from five of the six negative strains (BX232, NG3/88, NGP165, M136 & M986) and from three of the positive strains (8047, CU385 & NG6/88) were sequenced. The sequence for the sixth negative strain (MC58) was already available from the complete genome sequence.

The *nspA* sequences for all ten strains were highly conserved, varying at most by 5 nucleotides 30 from the prototype sequence of Martin *et al.* The most variant protein had only 3 amino acid differences (see Figure 29). With one exception, all of the amino acid variants involved the

same respective residues in discrete segments of the protein. These include the signal peptide, which is not present in the mature protein, and two short segments in the 50 C-terminal residues. These differences do not explain the antisera results, as there are examples of identical variant sequences in strains that were positive and those that were negative (compare M136 & 5 8047; NGP165 & NG6/88; MC58 & CU385).

As neither lack of the gene nor polymorphism explained the antiserum results, the amount of NspA protein in the outer membranes of five strains (8047, CU385 & NG6/88 – all positive for anti-rNspA; M986 & M136 – both negative) were tested. Bacterial cell pellets were extracted with lauryl sarcosinate, and the insoluble outer membrane fractions were analysed. An 18.6kDa 10 band was seen for all five strains, and this was cross-reactive with anti-His-tag-NspA by Western Blot. Thus strain differences in *nspA* expression also failed to explain the results.

The ability of anti-rNspA to bind to the bacterial cell surface could be influenced by the amount of polysaccharide capsule present. The quantity of capsular polysaccharide produced by the 17 test strains was therefore assessed by inhibition ELISA.

15 Extracts of capsular polysaccharide were prepared based on a method described by Corn *et al.* [*J.Infect.Dis.* (1993) 167:356-64]. Individual bacterial clones were grown to an OD₆₂₀ 0.5-0.7 in 7 mL of Mueller-Hinton broth. Bacteria were collected by centrifugation at 5000g for 15 min, washed in 0.6mL of 10 mM Hepes, pH 8.0, and then resuspended in 0.6 mL of the same buffer containing 10 mM EDTA and incubated at 37°C for 1 hr. The cells were pelleted at 10,000g for 20 1 minute and the relative amount of meningococcal B polysaccharide antigen released into the supernatant was determined by an inhibition ELISA, performed as described by Azmi *et al.* [*Infect. Immun.* (1995) 63:1906-13]. The solid phase antigen in the ELISA was meningococcal B polysaccharide-ADH-biotin absorbed to avidin-coated microtiter plates [Granoff *et al.*]. The meningococcal B polysaccharide-reactive human paraprotein LIP [Azmi *et al.*] was used as the 25 primary antibody (0.2 µg/ml). In the absence of inhibitor, this concentration of antibody was sufficient to give an OD of ~0.7 to 1.0 after 30 minutes incubation with substrate [Azmi *et al.*]. The titre of polysaccharide released into the supernatant was measured by determining the dilution of supernatant that resulted in 50% inhibition of antibody binding. Controls in this assay included an EDTA extract prepared from the strain M7, which does not produce any 30 capsular polysaccharide, and purified meningococcal B polysaccharide. To ensure that all of the capsular polysaccharide was released by the EDTA treatment, the same inhibition ELISA was performed using the cell pellet resuspended in the same buffer and volume as the capsule

extract. The observable inhibitory activity from the cell pellet was between 0 and 10% of the activity observed in the capsule extracts with the latter, higher percentage coming from cell pellets of strains that produce the largest amounts of capsule.

- The results for each strain are shown in the table on page 72. On average, the six negative anti-
5 rNspA strains produced three-fold more capsular polysaccharide than the eleven positive strains (respective reciprocal geometric mean dilutions of 676 vs. 224, $p<0.05$). This may explain the results obtained with the antiserum – conceivably, the presence of larger amounts of capsule could interfere with the ability of the anti-rNspA antibody to bind to NspA epitopes which, in strains with lower amounts of capsule, are accessible.
- 10 The complement-dependent bactericidal activity of the anti-rNspA antisera were tested using an assay similar to that described by Mandrell *et al.* [*J. Infect. Dis.* (1995) 172:1279-89]. The complement source was human serum from a healthy adult with no detectable anti-capsular antibody to group B polysaccharide and no intrinsic bactericidal activity against the test strain. Serum bactericidal titres were defined as the serum dilution resulting in a 50% decrease in
15 CFU/ml after 60 minutes incubation of bacteria in the reaction mixture, compared to the control CFU/ml at time zero.

- Typically, bacteria incubated with a negative control antibody showed a 150-200% increase in CFU/ml during the 60 minutes of incubation. The positive control antibody [anti-capsular IgG2a mAb SEAM 12, Granoff *et al.*] showed complement-mediated bactericidal activity
20 against all 17 strains. In contrast, the six strains that were negative for anti-rNspA antisera binding by flow assay were resistant, showing no bactericidal or bacteriostatic effects. Ten of the other eleven positive strains were either killed by complement and the antisera (SWZ107, J351, CU385, NG6/88, BZ198, H44/76, NMB & 8047) or were inhibited (H355 & S3446); strain 1000, however, was not affected.
- 25 The ability of the anti-rNspA antisera to confer passive protection against meningococcal B bacteremia was tested in infant rats using a method adapted from Saukkonen [*J. Infect. Dis.* (1988) 158:209-212]. Briefly, 6-7 day old rats were randomly distributed to nursing mothers. Groups of 5-6 animals were challenged IP with 100 μ l of approximately 5000 CFU of *N.meningitidis* group B bacteria. One strain negative for NspA surface epitopes (M986) and one
30 positive strain (8047) were tested, each of which having been passaged three times in infant rats. Immediately before administration, the bacterial suspension was mixed with different dilutions of test or control antibody (positive control: anticapsular mAb; negative control:

anti-*E.coli*). 18 hours after challenge, blood specimens were obtained from the heart. Aliquots were plated onto chocolate agar, and CFU/ml was determined after overnight incubation at 37°C in 5% CO₂.

The protective activities of the various co-administered antibodies were as follows:

Antibody treatment	Dose per rat or serum dilution	Strain	Blood culture		
			Positive/ total	CFU/ml (mean x10 ⁻³)	CFU/ml (% of control)
Anticapsular mAb	2µg	M986	0/6	<1	<1
Anti-rNspA	1:5	M986	6/6	44 ^a	45
Anti-rNspA	1:25	M986	6/6	93 ^a	95
Anti- <i>E.coli</i> control	1:5	M986	6/6	98 ^a	-
Anticapsular mAb	2µg	8047	0/5	<1	<1
Anti-rNspA	1:5	8047	1/6	0.2 ^b	2
Anti-rNspA	1:25	8047	1/5	0.4 ^b	4
Anti- <i>E.coli</i> control	1:5	8047	6/6	10 ^b	-

5 ^a p>0.5, compared to geometric mean CFU/ml of control rats

^b p<0.001, compared to geometric mean CFU/ml of control rats

As can be seen, a dose of 2µg per rat of the positive anticapsular control was protective against both strains. A 1:5 or 1:25 dilution of anti-rNspA antiserum protected against bacteremia caused by strain 8047. Neither dilution was effective in preventing M986 bacteremia, however.

- 10 Despite the positive conclusions of Martin *et al.*, therefore, NspA does not seem to be effective in preventing meningococcal B infection. Approximately one third of strains have decreased cell-surface expression of NspA epitopes when grown *in vitro*, are resistant to anti-NspA induced complement-mediated bacteriolysis, and are resistant to passive antiserum immunisation. These strains produce large amounts of capsular polysaccharide, and would thus
- 15 be expected to have the greatest virulence. The ability of a vaccine containing only NspA to confer broad protective immunity against meningococcal B thus has to be doubted.

Compositions comprising NspA [SEQ IDs 4008-4033; Figure 29] therefore advantageously comprise further antigens. A preferred aspect of the invention is thus a combination of NspA protein with one or more further Neisserial antigens.

Example 12 – NspA fragments

A model of the secondary structure of NspA is shown in Figure 32, containing eight transmembrane β -strands and 4 surface-exposed connecting loops. This fits the pattern of alternating hydrophobic and hydrophilic amino acids in NspA, which is characteristic of many
5 β -barrel porins [Weiss *et al.* (1990) *FEBS Letts* 267:268-272]

The grey shaded areas in the model indicate segments that are >40% identical and >70% similar to encoded amino acid sequences of opacity proteins (Opa) from *N.meningitidis*, *N. gonorrhoeae*, *N. flavius*, *N. sicca*, and *H. influenzae* identified in a BLAST search of the non-redundant GenBank CDS. The alternating sequences are predicted amphiphilic β -strands;
10 vertical segments correspond to transmembrane segments; the top of the figure corresponds to surface exposed segments, labelled as loops 1 to 4 .

According to Martin *et al.*, the only significant homology between the deduced amino acid sequence of NspA and those of other proteins are weak homologies with the Neisseria opacity protein (Opa) family in two small segments (~20 amino acids) near the C-terminal end of the
15 protein . However, separate comparisons of the N- and C-termini of NspA with GenBank reveals a high degree of homology (>40 % identity and >70% similarity) between NspA and Opa proteins from *N.meningitidis*, *N. gonorrhoeae*, *N. flavius*, *N. sicca*, and *H. influenzae*. The Opa proteins are thought to be integral membrane proteins that have eight transmembrane segments and a β -barrel topology in the membrane similar to that of porin [Merker *et al.* (1997)
20 *Mol. Microbiol.* 23:281-293]. The presence of NspA in detergent-insoluble membrane preparations indicate that NspA is located in the outer membrane, which would be consistent with the Opa-like membrane topology shown in the model. In addition, the segments of NspA that are most homologous to those of the Opa proteins are the putative transmembrane segments indicated in the shaded areas of Figure 32.
25 The opacity proteins of Neisseria can, under certain circumstances, elicit protective antibody. However, problems with limited antibody accessibility of the opacity proteins in encapsulated bacteria, variability of amino acid sequences in exposed loop segments, and phase variation of protein expression during clinical infection, have limited the ability of Opa to elicit protective antibody consistently [Malorny *et al.* (1998) *J. Infect. Dis.* 172:1279-89]. In contrast, there
30 appears to be little or no sequence variation in the surface exposed loops of NspA in Figure 32. However, it was recently reported that a panel of anti-*N.meningitidis* NspA monoclonal antibodies that reacted with all meningococcal strains tested reacted with only a limited number

of *N.gonorrhoeae* strains, even though the respective amino acid sequences in the two species are 92% identical. When the respective NspA sequences of the meningococcal and gonococcal strains are compared (Figure 29), all of the respective amino acid differences result in changes in hydrophobicity or charge, and are located in the putative surface exposed connecting loops 5 (Figure 32). This finding suggests that the connecting loops in NspA, which are highly conserved in *N.meningitidis*, may be important epitopes for antibodies that bind to native NspA. These segments of the molecule, therefore, would appear to be of greatest interest with respect to interacting with protective antibody. However, the putative surface loops of NspA are relatively small (10-14 amino acids) compared to, for example, the highly immunogenic 10 external loops of PorA and Opc (24 to 45 amino acids). The shorter length of the loops may limit the accessibility of NspA surface epitopes for binding interactions with serum antibody, especially in the presence of abundant capsular polysaccharide.

Accordingly, the invention provides the fragments of NspA that are exposed on the cell-surface in Figure 32, namely SSSLGSAKG, NYKAPSTDFKLY, NRASVDLGGSDSFSQT, and 15 NYIGKVNTVKNVRSG, and also provides corresponding fragments from allelic variants of NspA. In addition, the invention provides sub-sequences of these fragments, comprising 7 or more contiguous amino acids from the fragments. The invention further provides proteins comprising these fragments. Nucleic acid encoding these fragments and proteins is also provided.

These NspA fragments, proteins comprising the fragments, and nucleic acid, may be used in the 20 compositions of the invention, in particular as substitutes for full-length NspA. In a further aspect, these fragments, proteins and nucleic acids may be used as products in isolation, that is to say they need not exclusively be used in combination with other biological molecules.

It will be appreciated that the invention has been described by means of example only, and that 25 modifications may be made whilst remaining within the spirit and scope of the invention.

Reactivity of anti-rNspA polyclonal antisera with native NspA exposed on the surface of live, encapsulated, *Neisseria meningitidis* B bacteria in relation to susceptibility to bacteriolysis and capsular production.

Strain	Country	Year	Serologic Classification	Meningococcal B Strains		NspA Cell Surface Reactivity ^c	Anti-rNspA Bactericidal Activity ^d (1/titer)	Polysaccharide Capsule Production (1/titer + SE)
				ET Complex	Reactivity ^c			
SWZ107 ^a	Switzerland	1980	4:P1.2	104	Positive	>64	28±4	
NG6/88 ^a	Norway	1988	NT: P1.1	173	Positive	4	115±23	
CU385 ^b	Cuba	1980	4: P1.15	5	Positive	4	116±1	
IH5341	Finland	1985	15:P1.7,16	ND	Positive	16	176±61	
BZ198	Netherlands	1986	NT: P-	154	Positive	>64	362±1	
NMB	US	1968	2b:P1.2,5	ND	Positive	16	244±20	
8047	US	1978	2b: P1.2	ND	Positive	16	1125±50	
H44/76	Norway	1976	15: P1.7, 16	5	Positive	24	99±16	
1000 ^a	USSR	1989	NT:P1.5	61	Positive	<4	287±12	
S3446 ^b	US	1972	14: P1.23, 14	11 (A1 cluster)	Positive	<4 (static=16)	585±151	
H355 ^b	Norway	1973	15: P1.5	11 cluster	Positive	<4 (static=4)	656±141	
BZ232 ^a	Netherlands	1964	NT:P1.2	76	Negative	<4	1493±18	
NG3/88 ^a	Norway	1988	8: P1.1	A4 cluster	Negative	<4	498±105	
MC58	UK	1985	15:P1.7,16b	5	Negative	<4	627±121	
M136 ^b	US	1968	11: P1.5	D1 cluster	Negative	<4	1056±81	
M986 ^b	US	1963	2a: P1.5, 2	B2 cluster	Negative	<4	1442±206	
NGP165	Norway	1974	NT:P1.2	37	Negative	<4	138±6	

^a Denotes strains that have been characterized further by multilocus sequence typing [Maiden, 1998].

^b Denotes strains obtained from the Frasch collection, US FDA. 8047 was obtained from W. Zollinger, Walter Reed Army Institute of Research, Washington, D.C. MC58 is the strain selected by TIGR for genomic sequencing. J351 was obtained from M. Sarvas, National Public Health Institute, Helsinki, Finland. The remaining strains are from the collection described by Seiler *et al.* [Seiler, 1996]. ET data are from Caugant *et al.* [J. Infect. Dis. (1990) 162:867-874], and Seiler *et al.*

^c By indirect fluorescence flow cytometry with anti-rNspA antisera.

^d Dilution of anti-rNspA antisera that when incubated for 60 min. with bacterial cells and 20% human complement yielded >50% decrease in CFU/ml, compared to that at time 0. "Static" refers to strains that were inhibited but not killed in the assay ($\geq 50\%$ but $< 100\%$ survival at 60 mins).

^e Titre defined as dilution of capsule extract giving 50% inhibition of antibody binding to meningococcal B polysaccharide antigen in an ELISA.

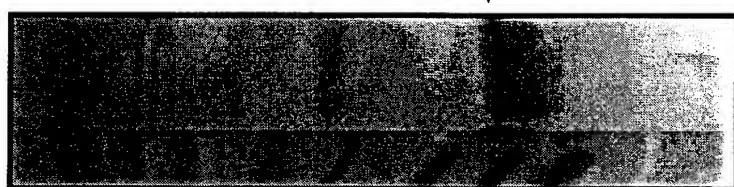
CLAIMS

1. A composition comprising a first biological molecule from a *Neisseria* bacterium and a second biological molecule from a *Neisseria* bacterium.
2. The composition of claim 1, wherein the first biological molecule is selected from the group consisting of SEQ IDs 1-8376.
5
3. The composition of claim 2, wherein the first biological molecule is selected from the group consisting of SEQ IDs 1-4002.
4. The composition of any preceding claim, wherein the second biological molecule is selected from the group consisting of SEQ IDs 1-8376.
10
5. The composition of claim 4, wherein the second biological molecule is selected from the group consisting of SEQ IDs 1-4002.
6. The composition of any one of claims 1 to 4, comprising two or more biological molecules selected from SEQ IDs 1-4002.
7. The composition of any one of claims 1 to 4, comprising one or more biological molecules selected from SEQ IDs 1-4002 combined with one or more biological molecules selected from SEQ IDs 4003-8376.
15
8. The composition of any one of claims 1 to 4, comprising one or more biological molecules selected from SEQ IDs 1-4002 & 4057-8376 combined with the NspA protein disclosed in WO96/29412, preferably in mature form.
9. The composition of any one of claims 1 to 4, comprising one or more biological molecules selected from SEQ IDs 1-8376 combined with 953 protein.
20
10. The composition of any preceding claim for use as a medicament

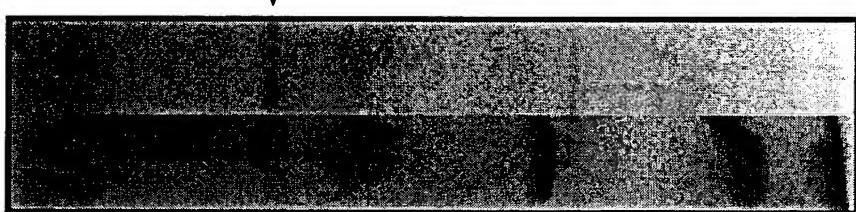
1/51



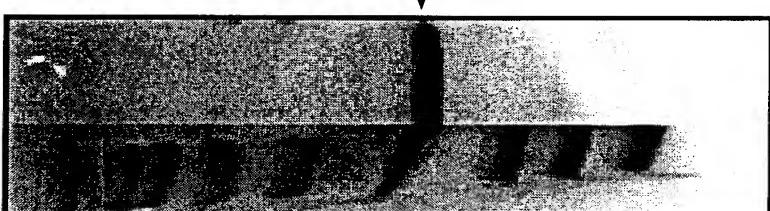
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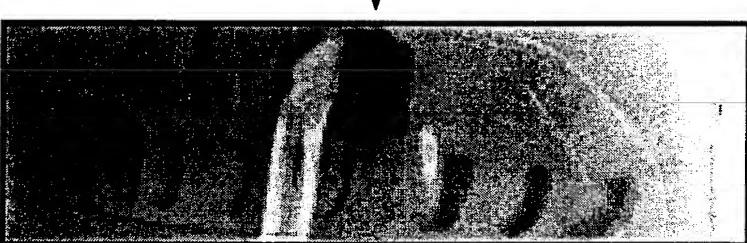
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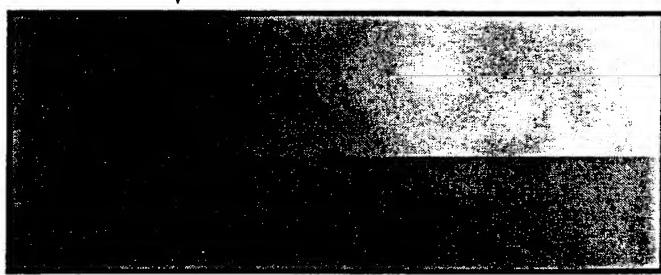
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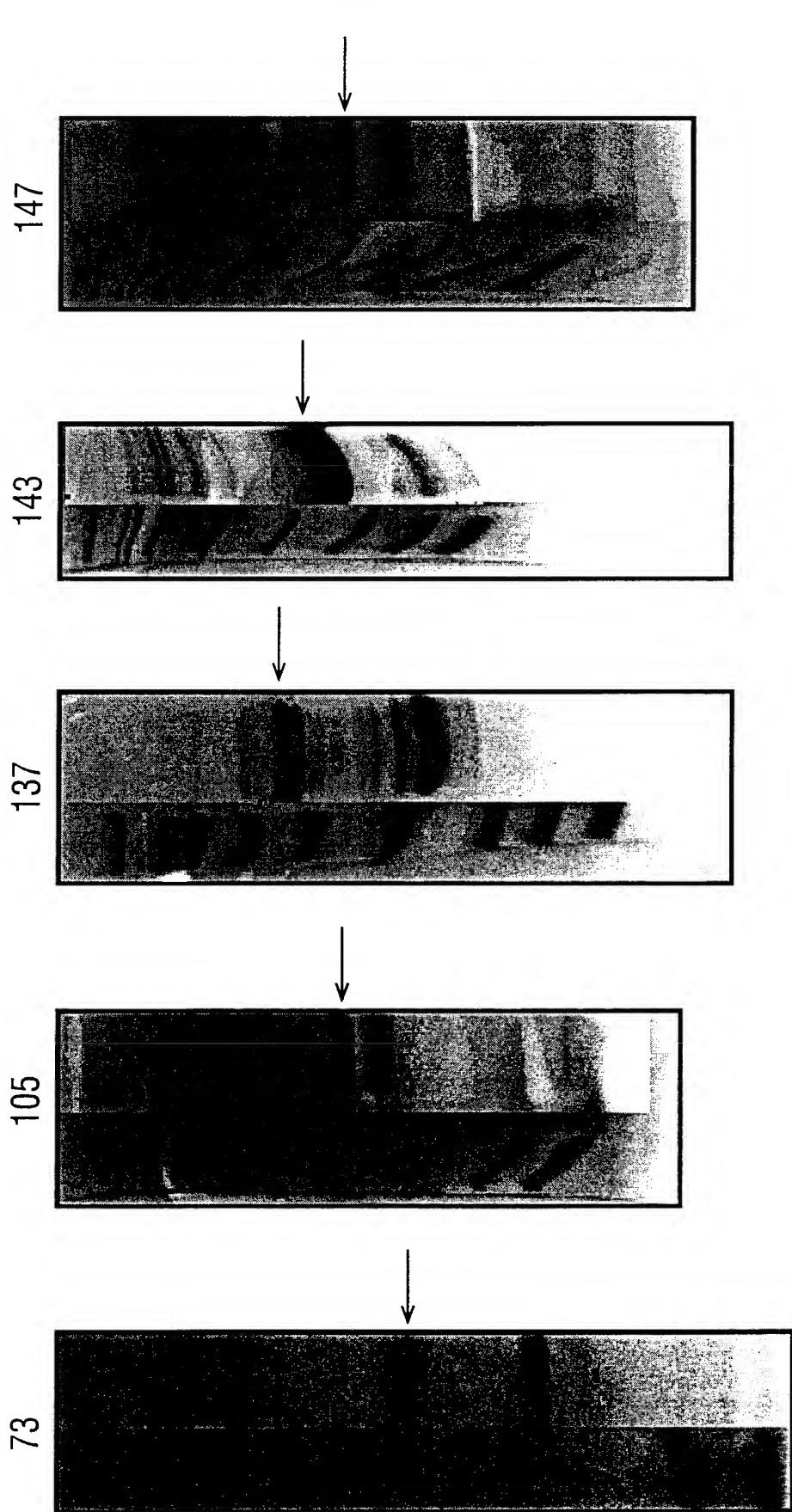


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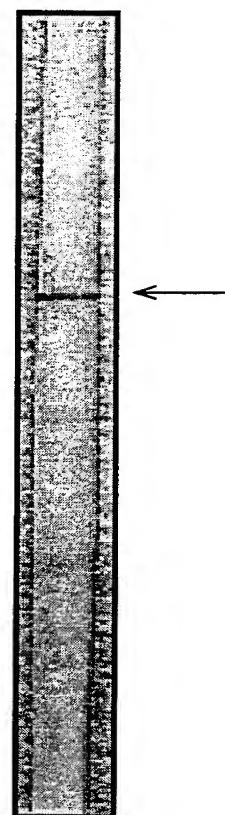
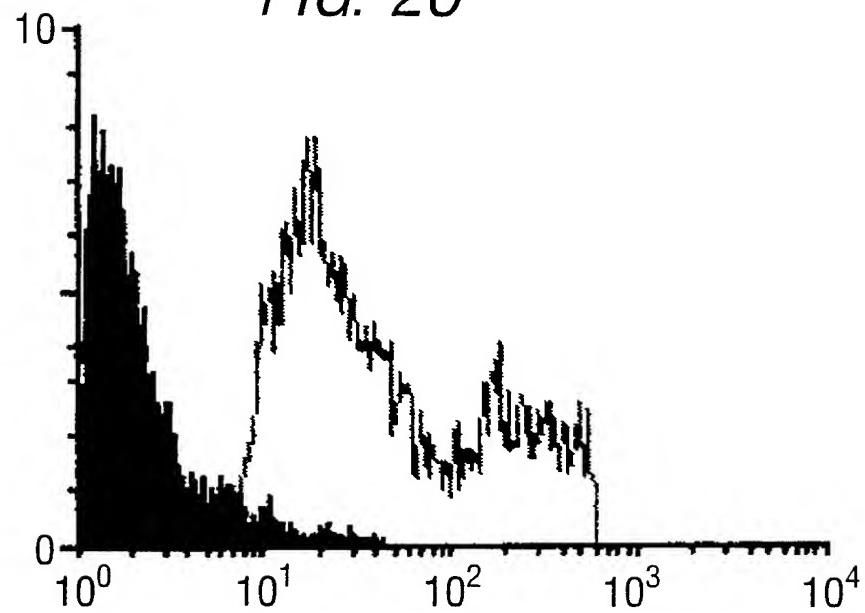
FIG. 1

2/51

FIG. 1 (CONT'D.)



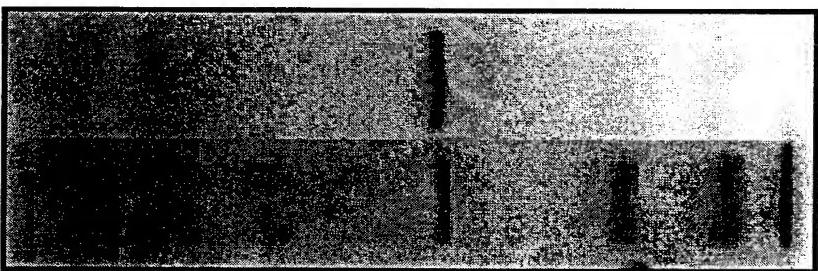
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FIG. 2A*FIG. 2B**FIG. 2C*

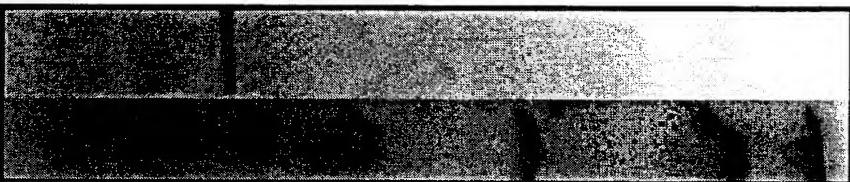
4/51



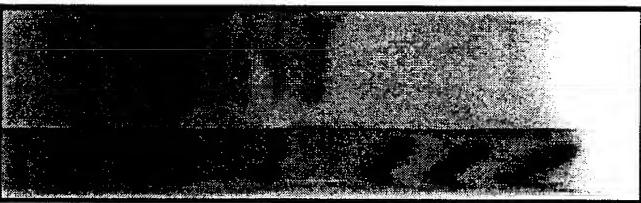
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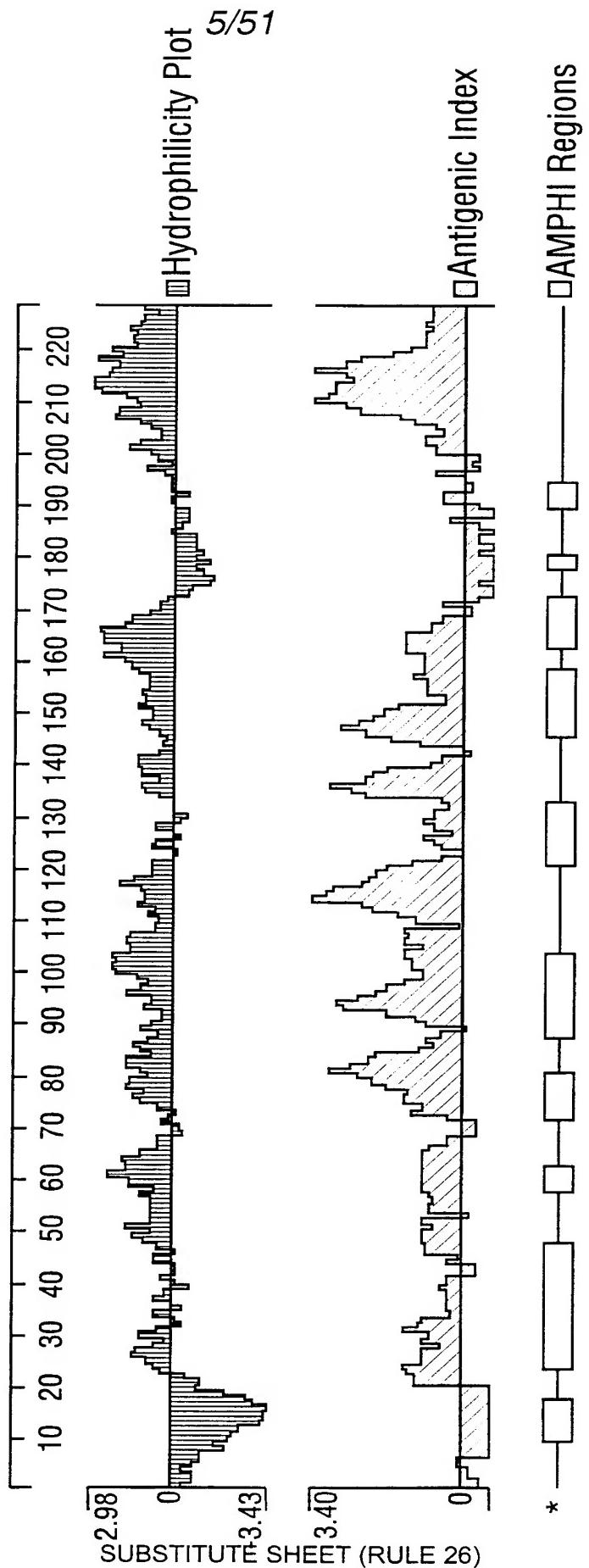
2



FIG. 3

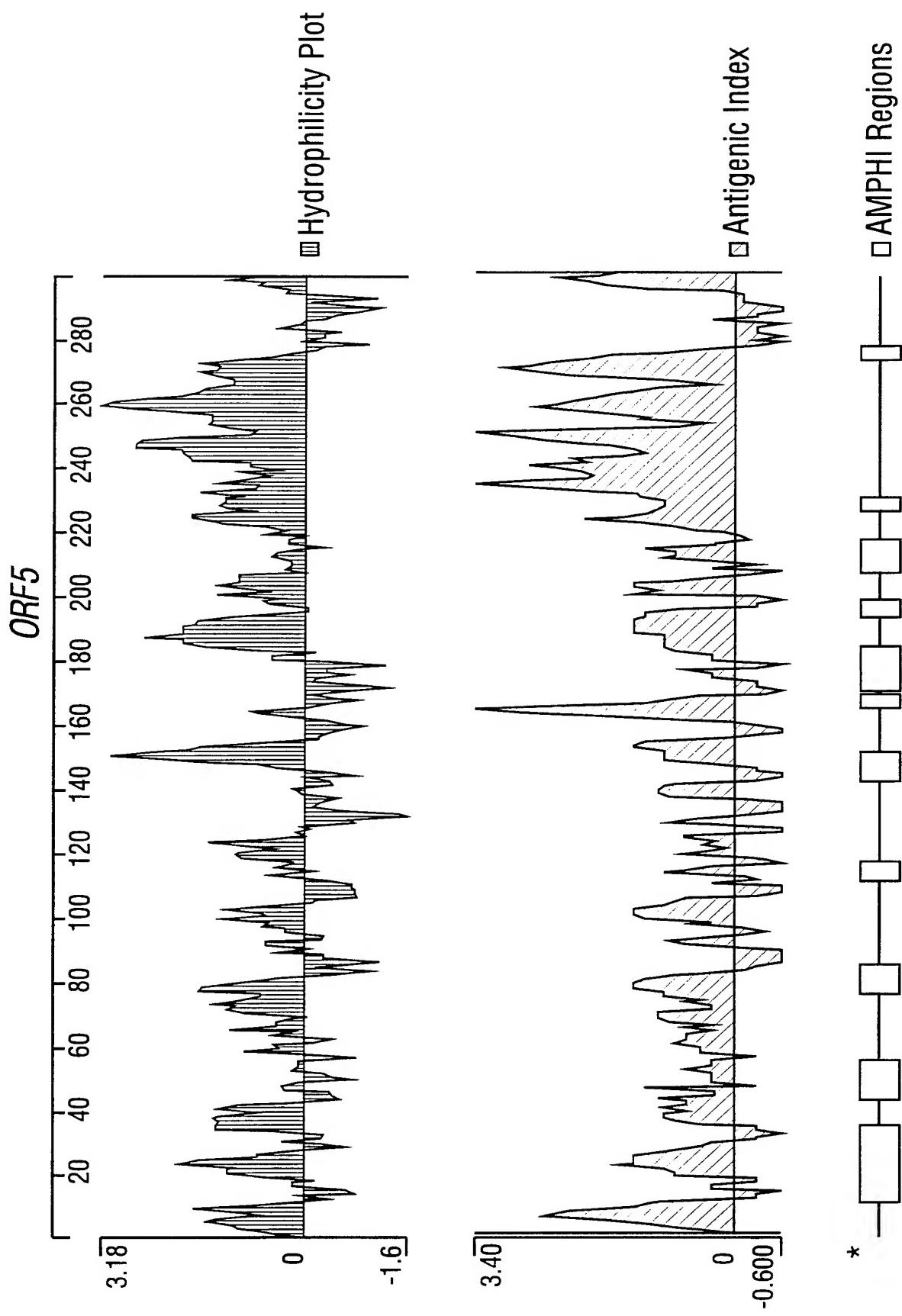
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FIG. 4
ORF2



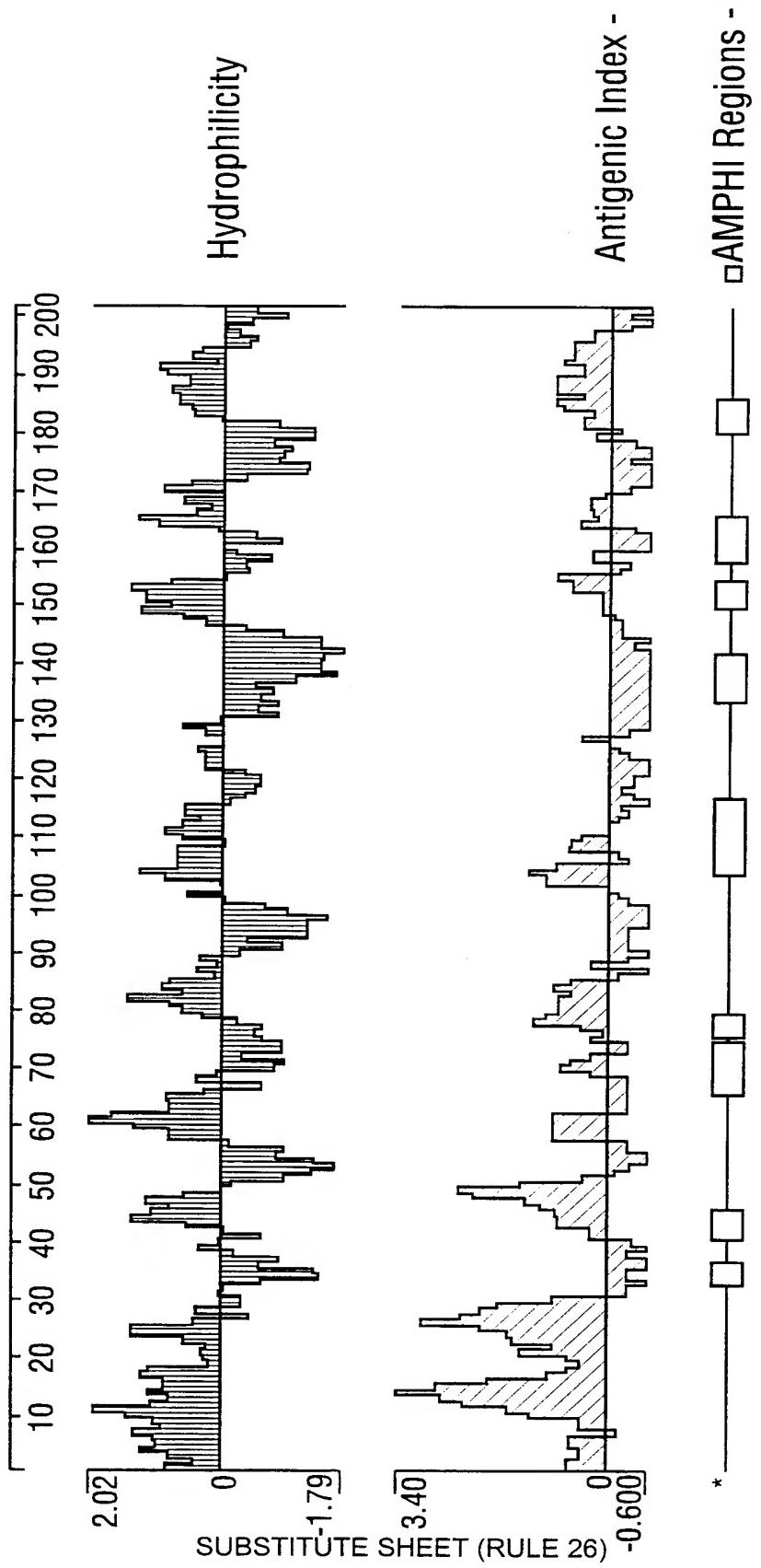
6/51

FIG. 5

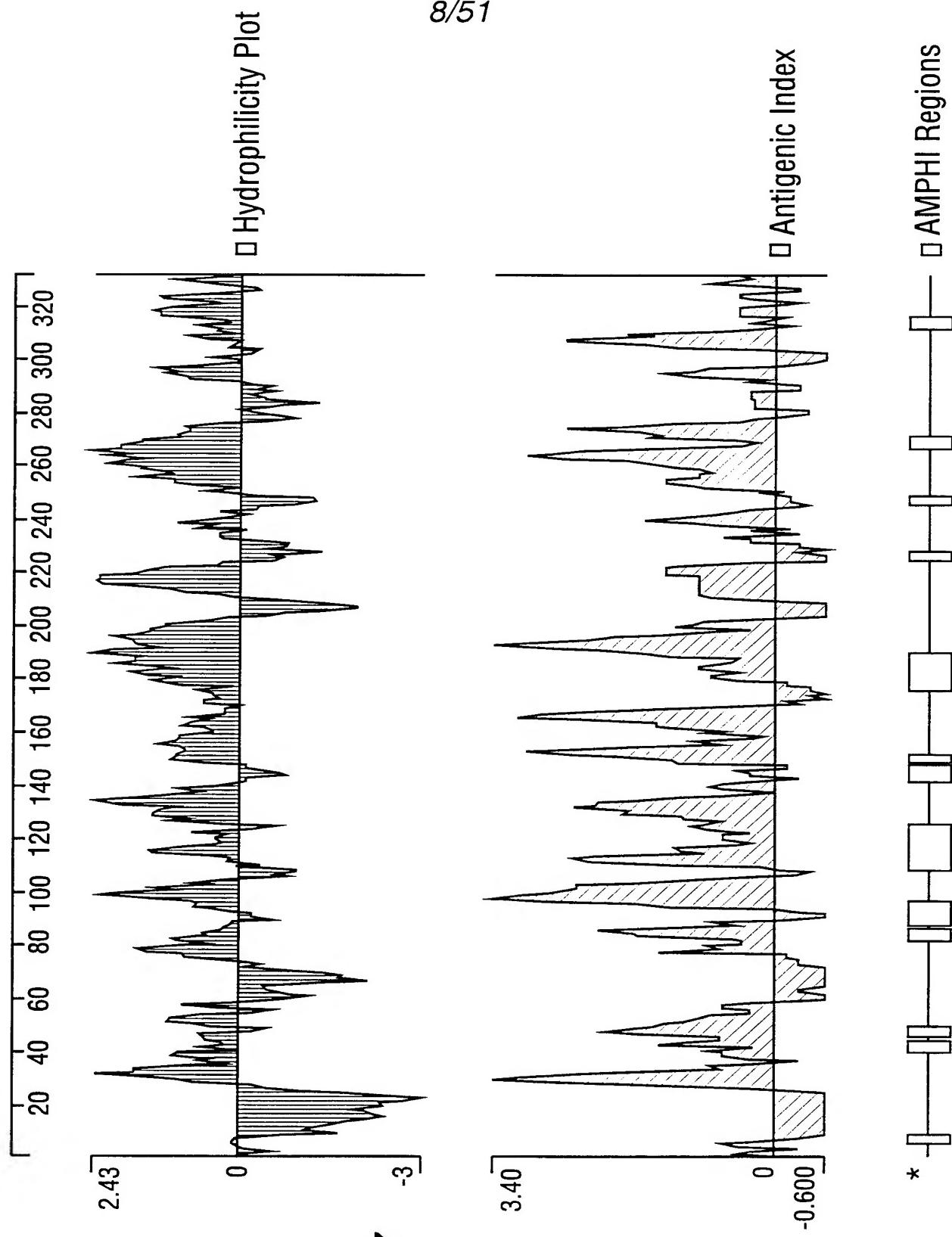


7/51

FIG. 6
ORF6a



8/51

FIG. 7
ORF7

9/51

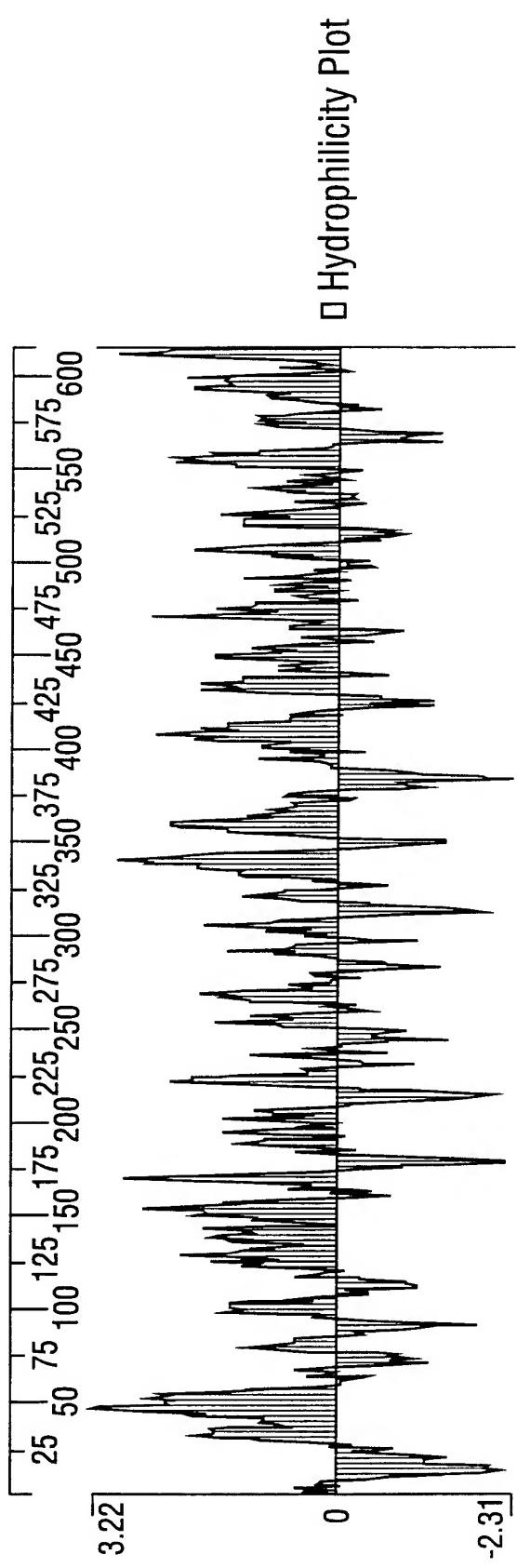
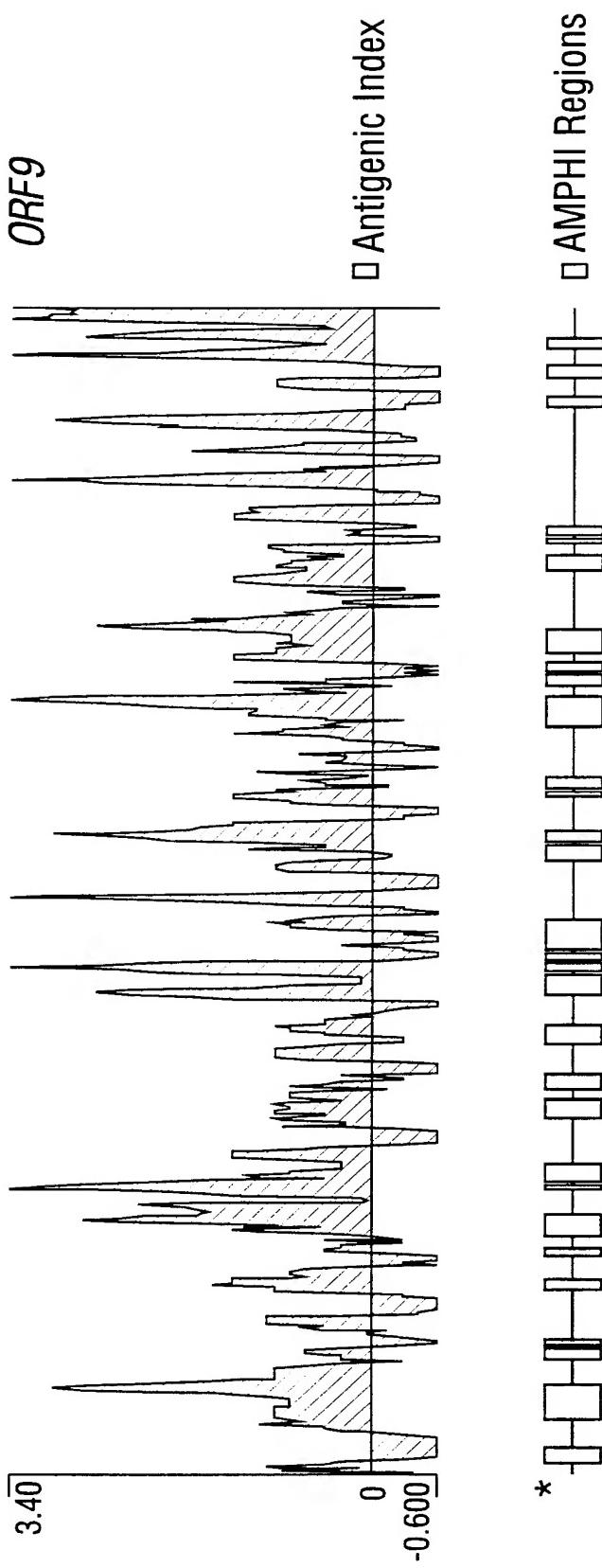
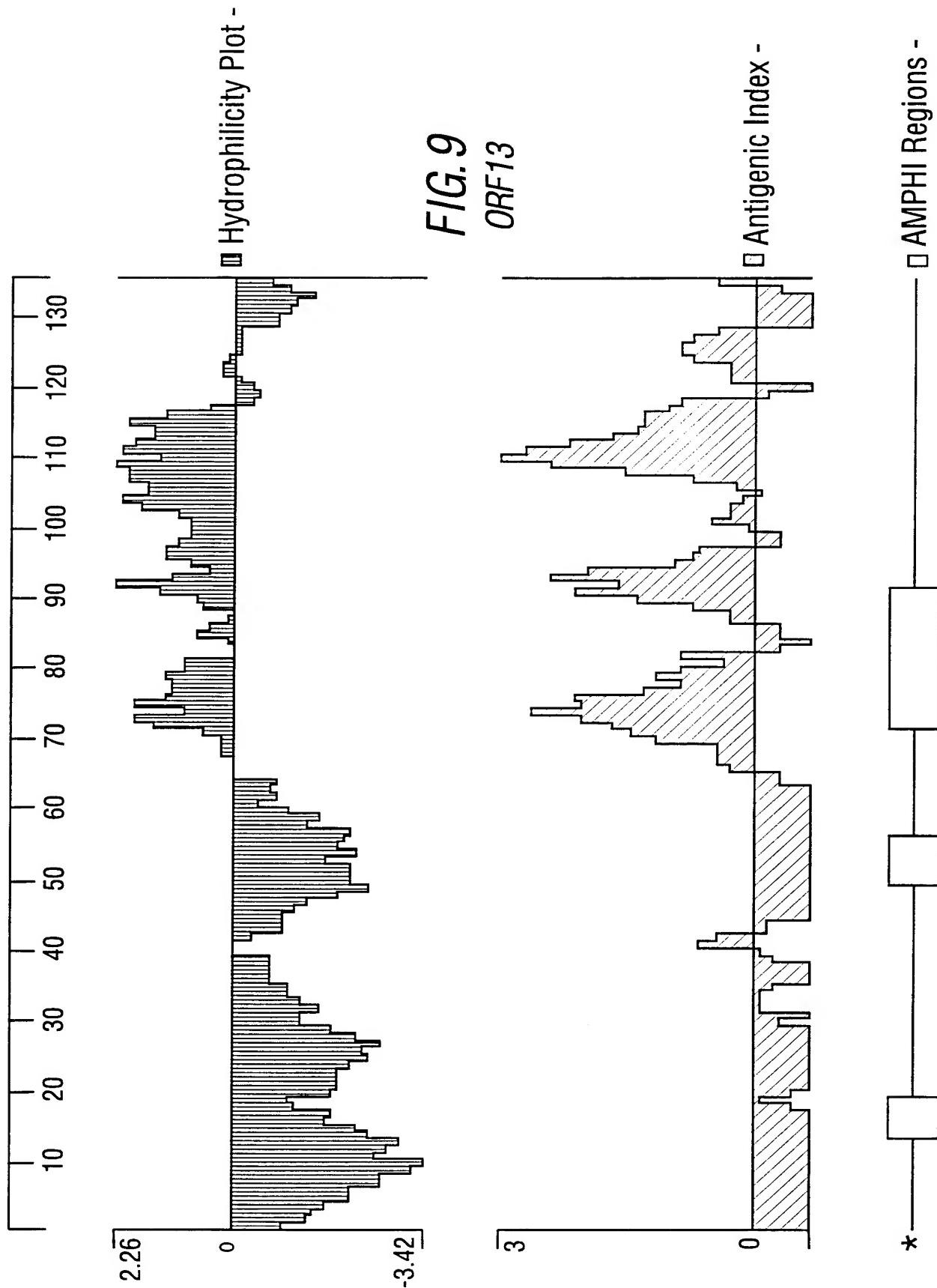


FIG. 8
ORF9



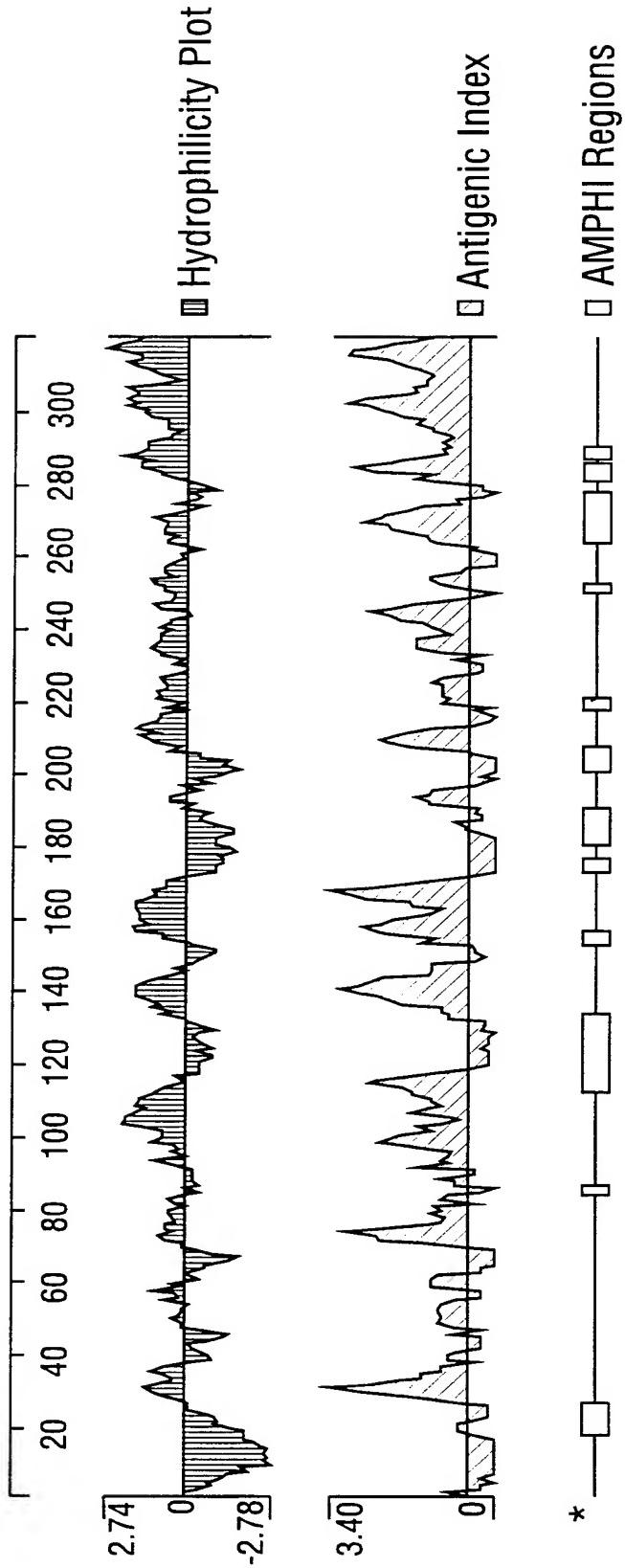
10/51

FIG. 9
ORF13



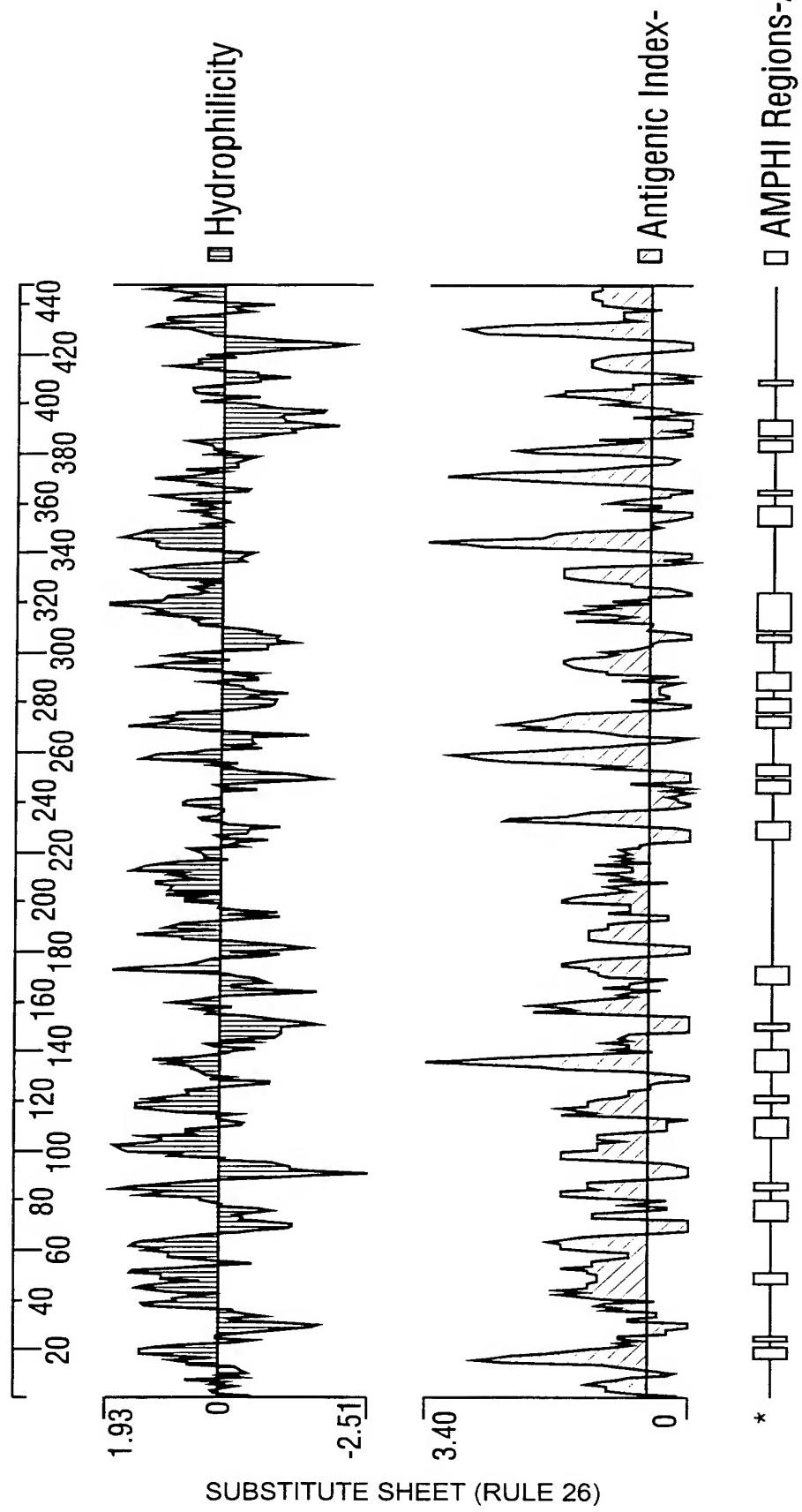
11/51

FIG. 10
ORF15

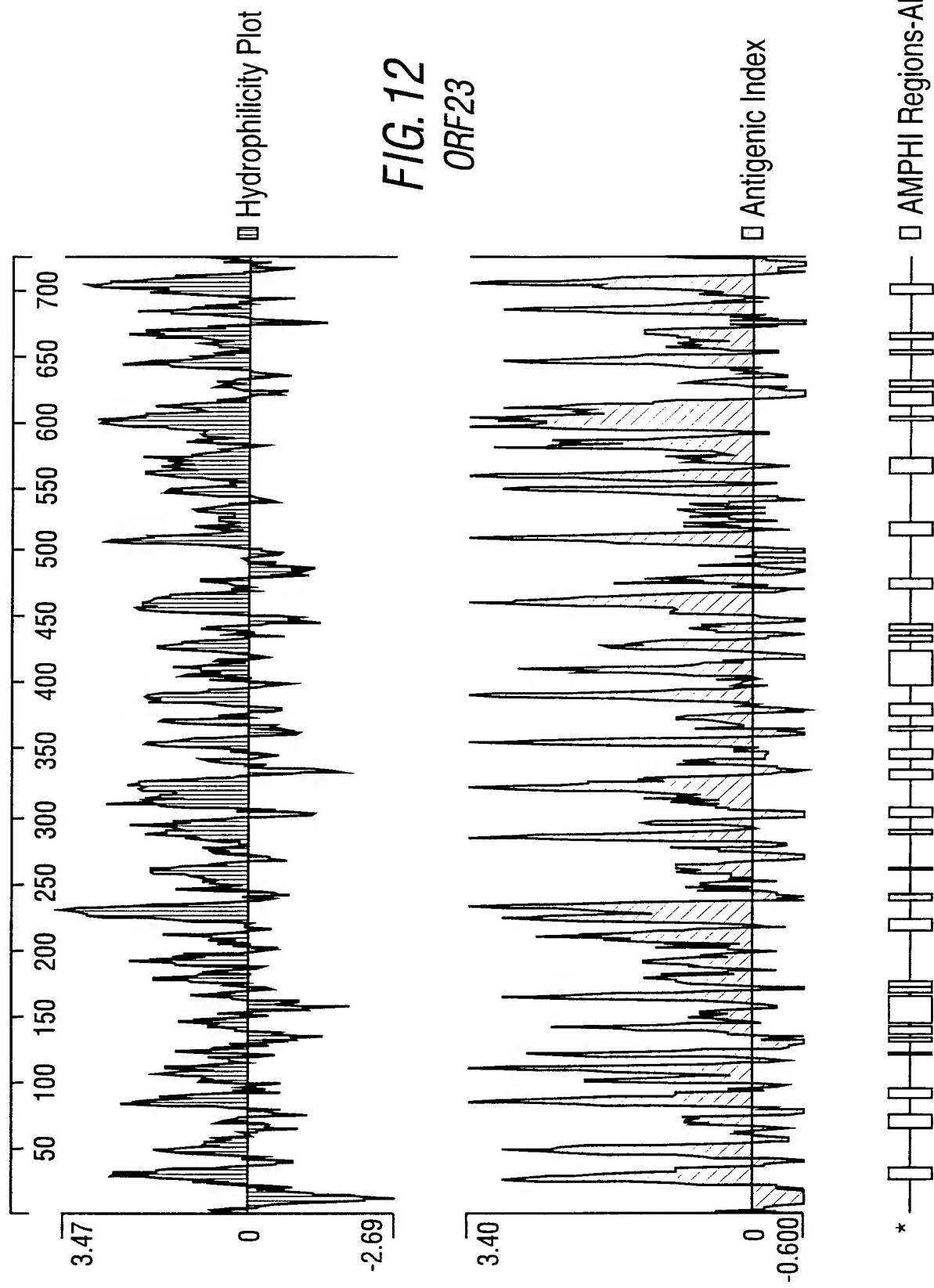


12/51

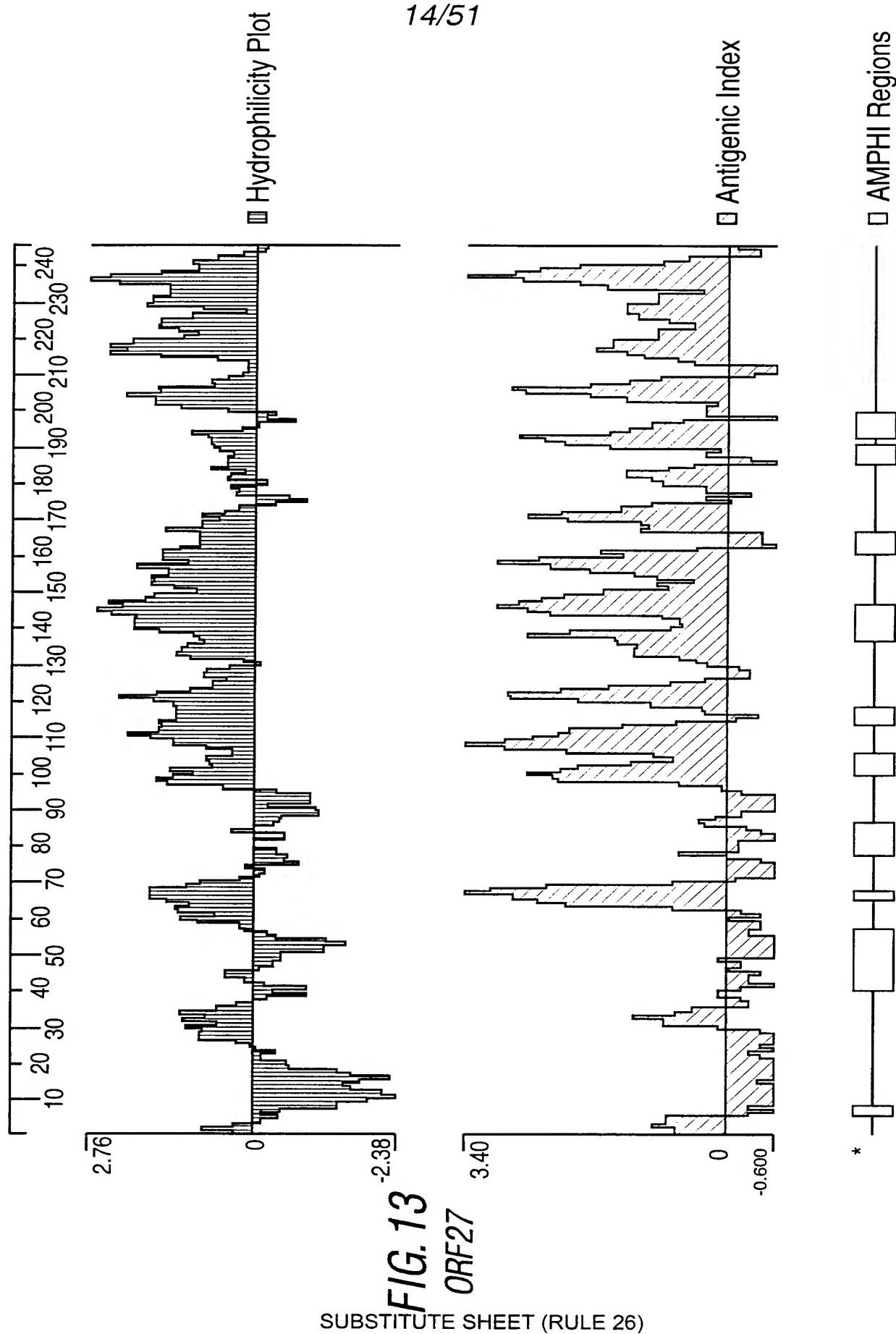
FIG. 11
ORF22



13/51

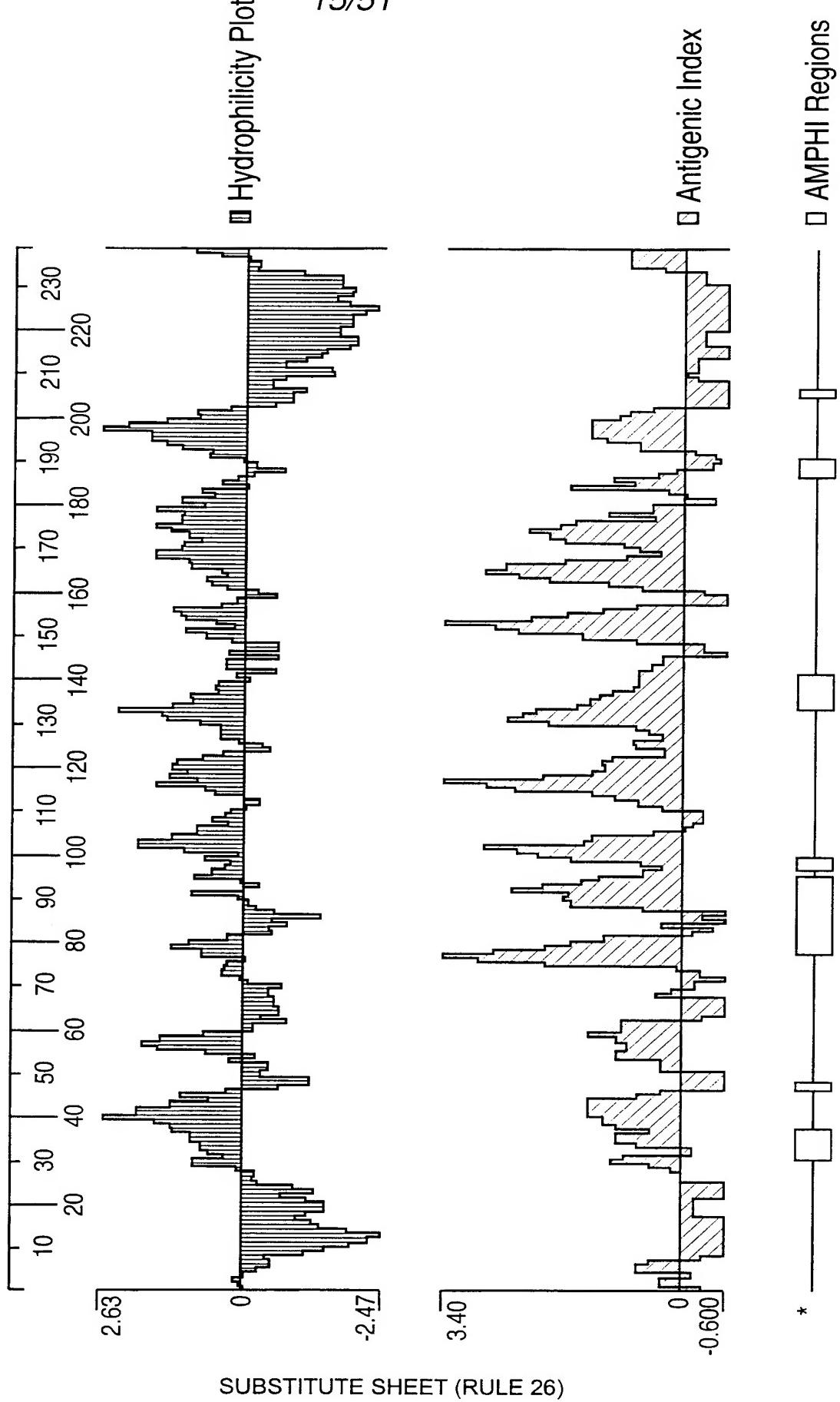


14/51



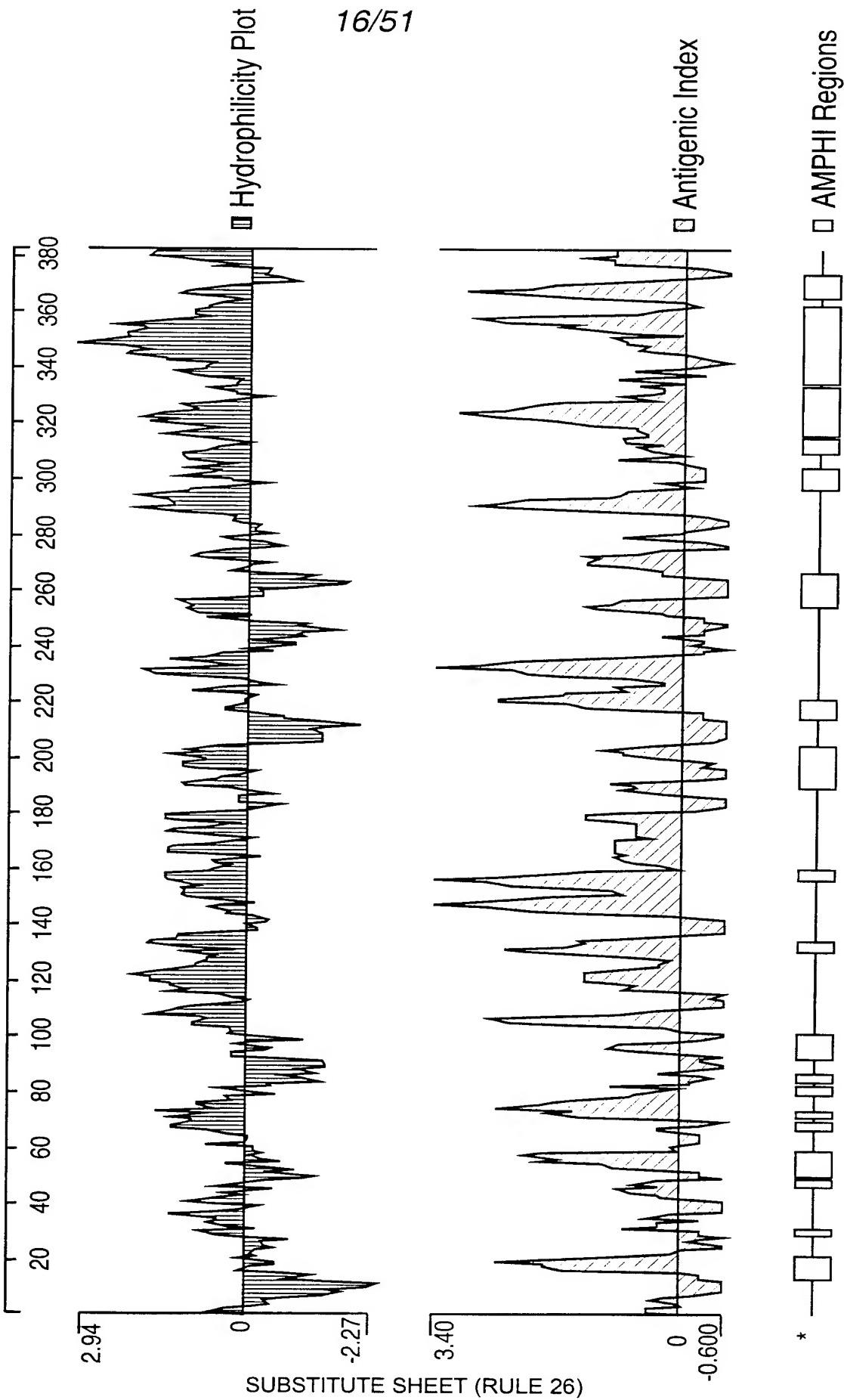
15/51

FIG. 14
ORF28



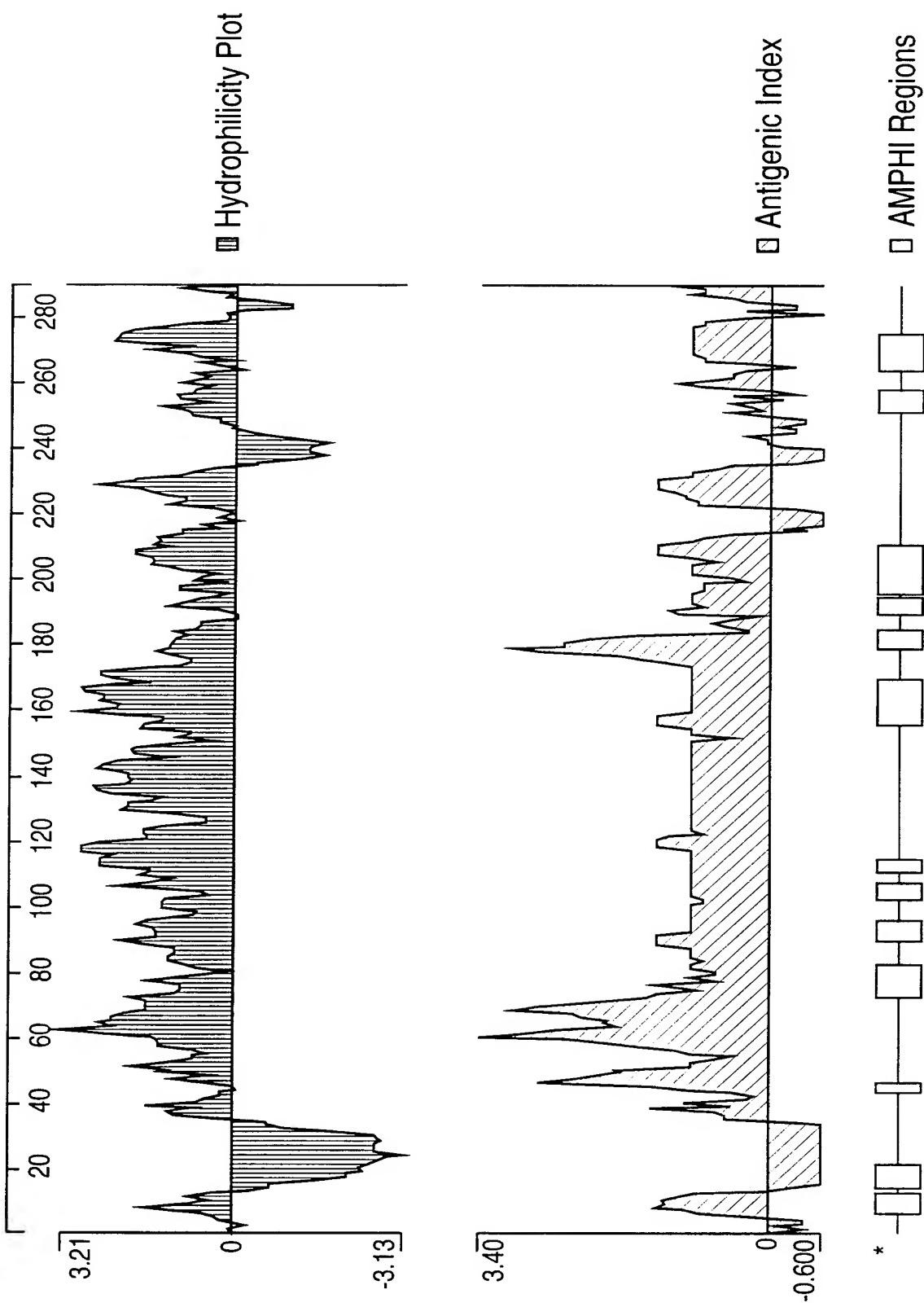
16/51

FIG. 15
ORF32



17/51

FIG. 16
ORF65



18/51

FIG. 17
ORF72

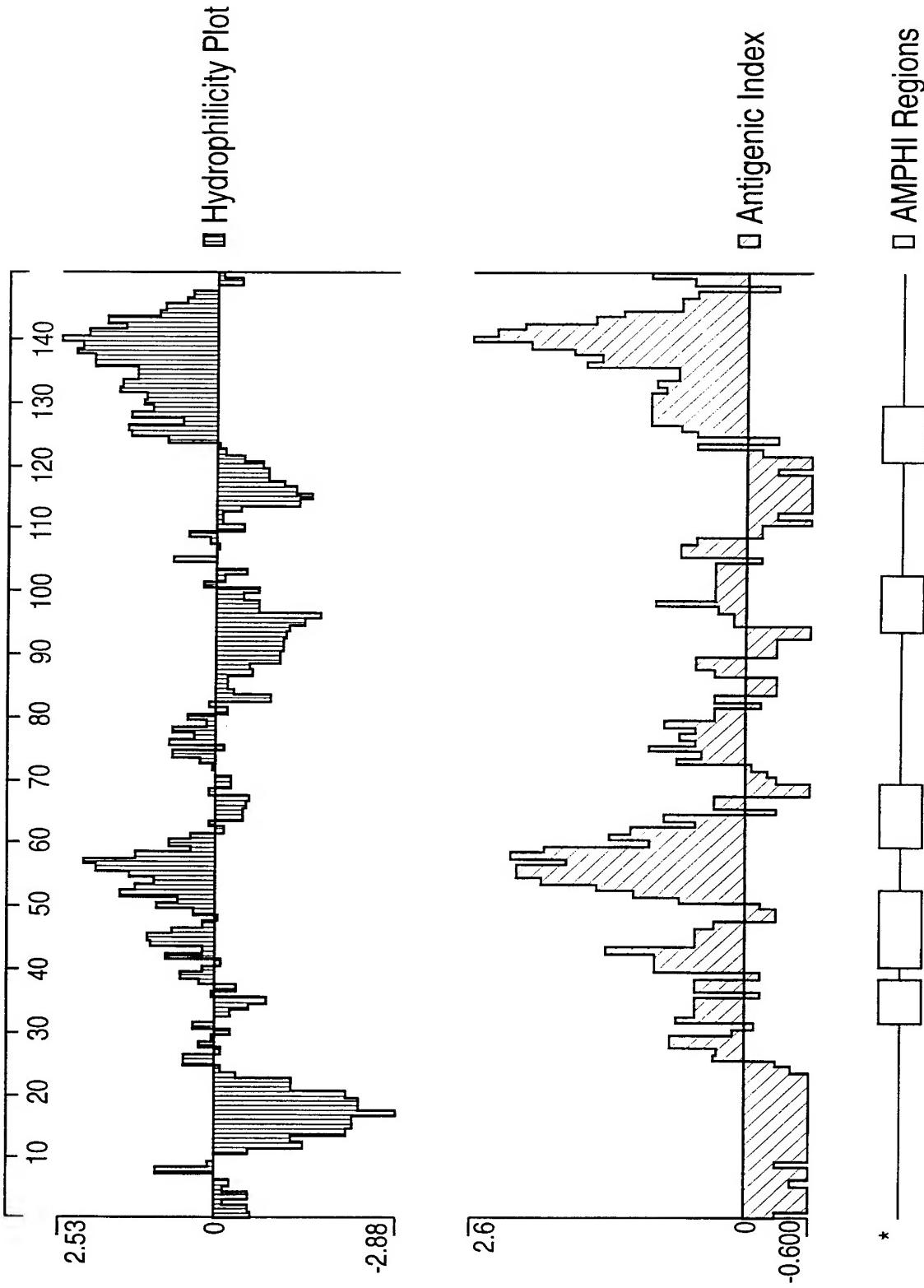


FIG. 18
ORF73

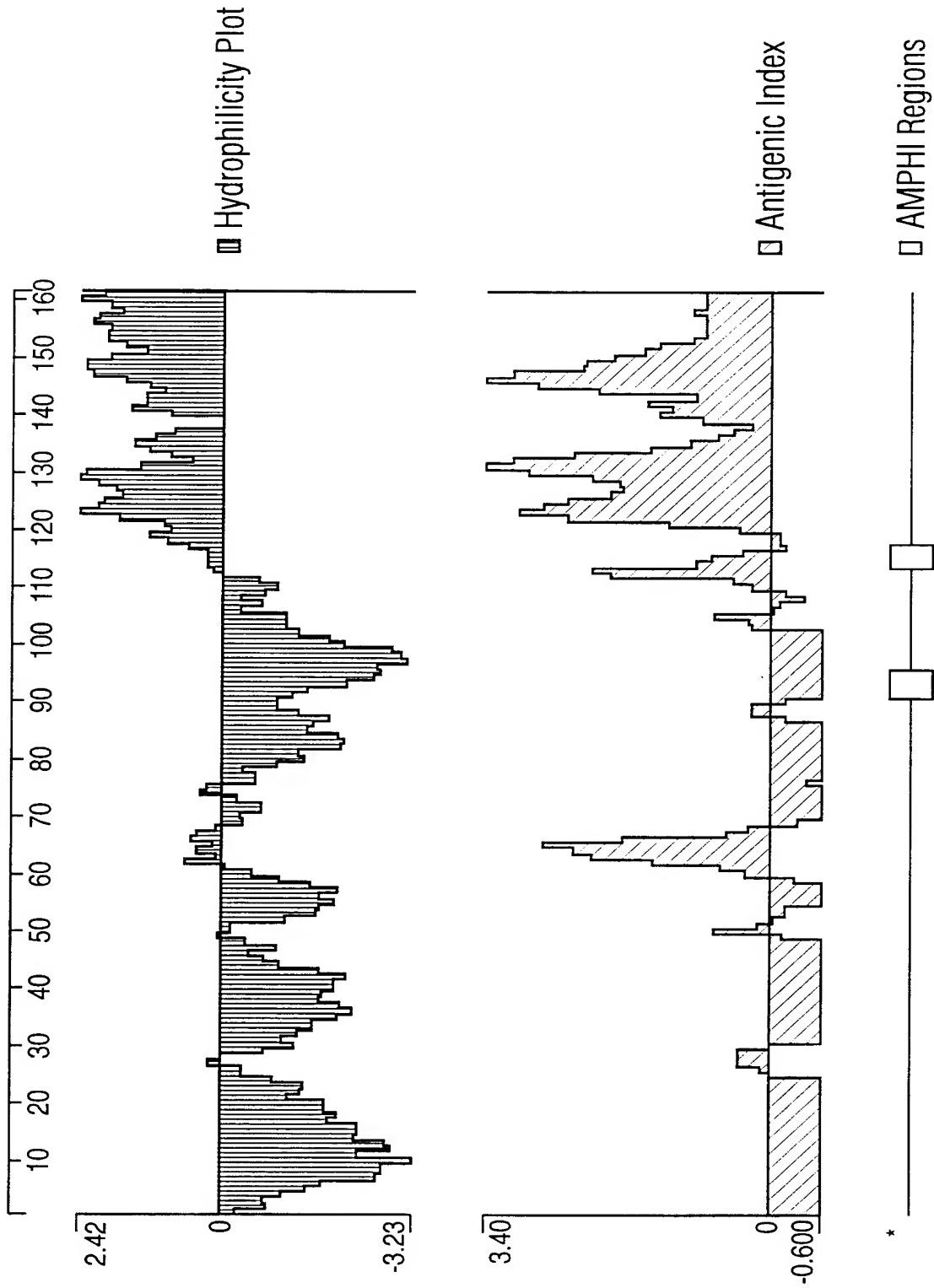


FIG. 19
ORF76

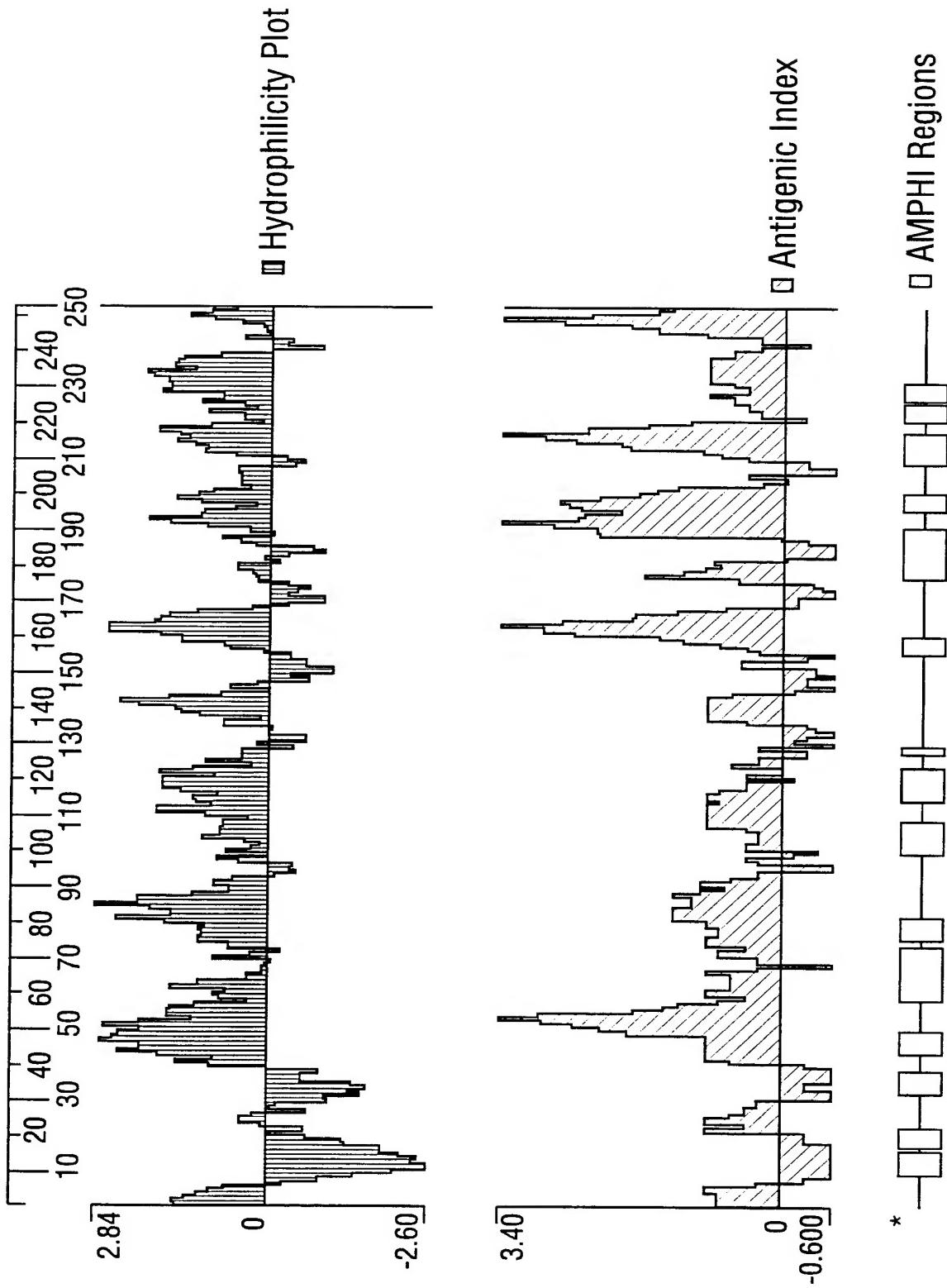


FIG. 20
ORF79

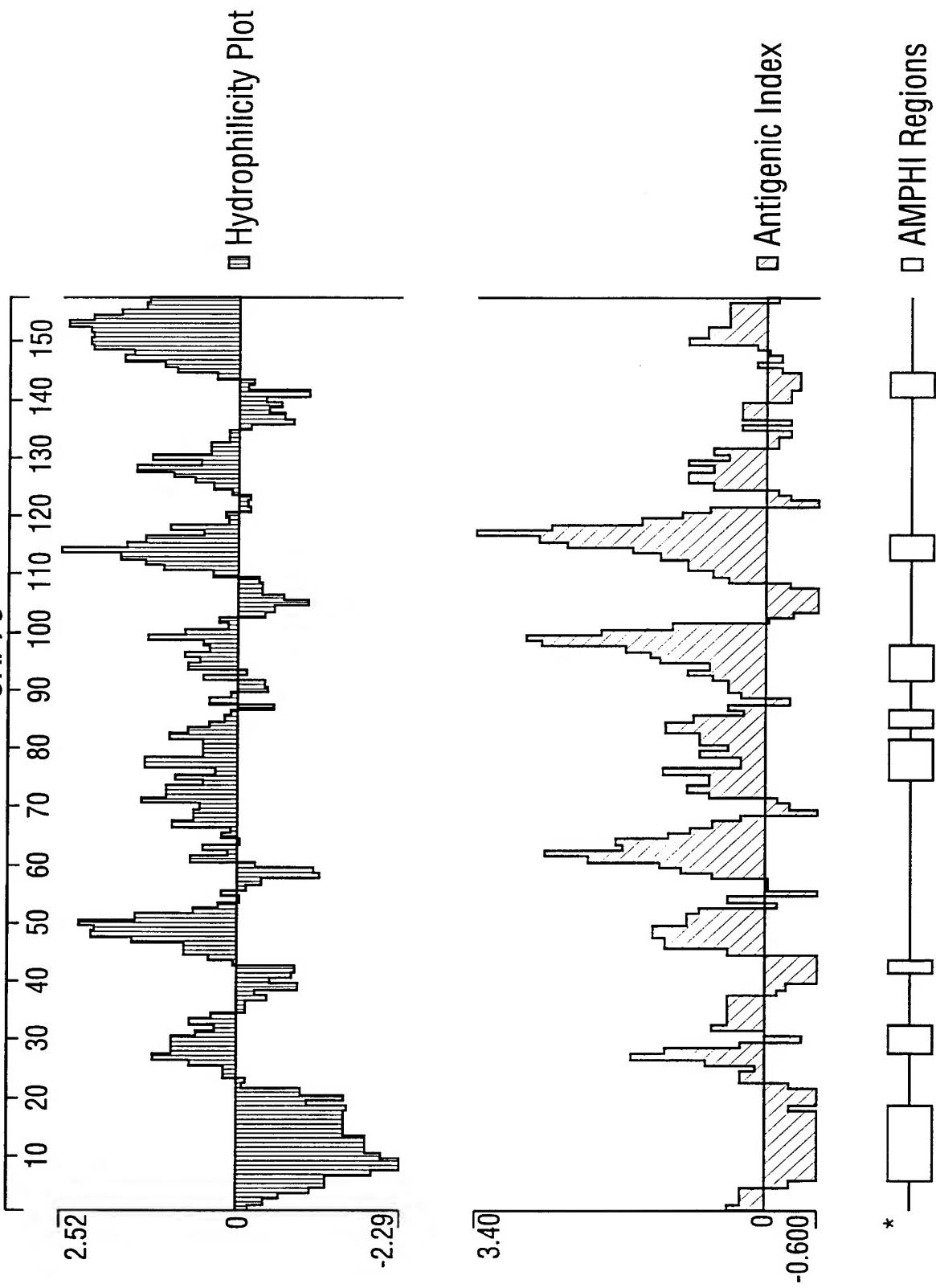
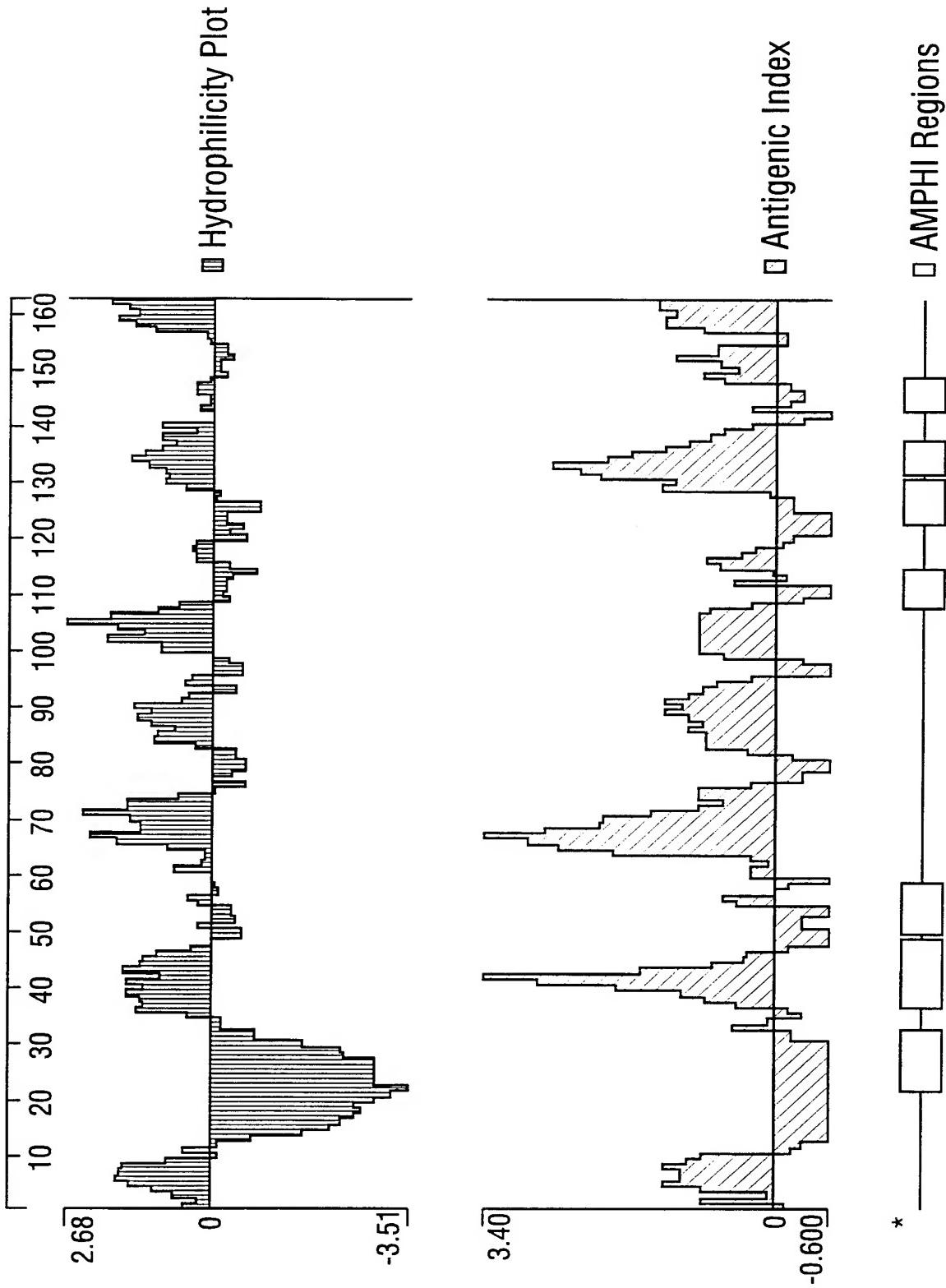
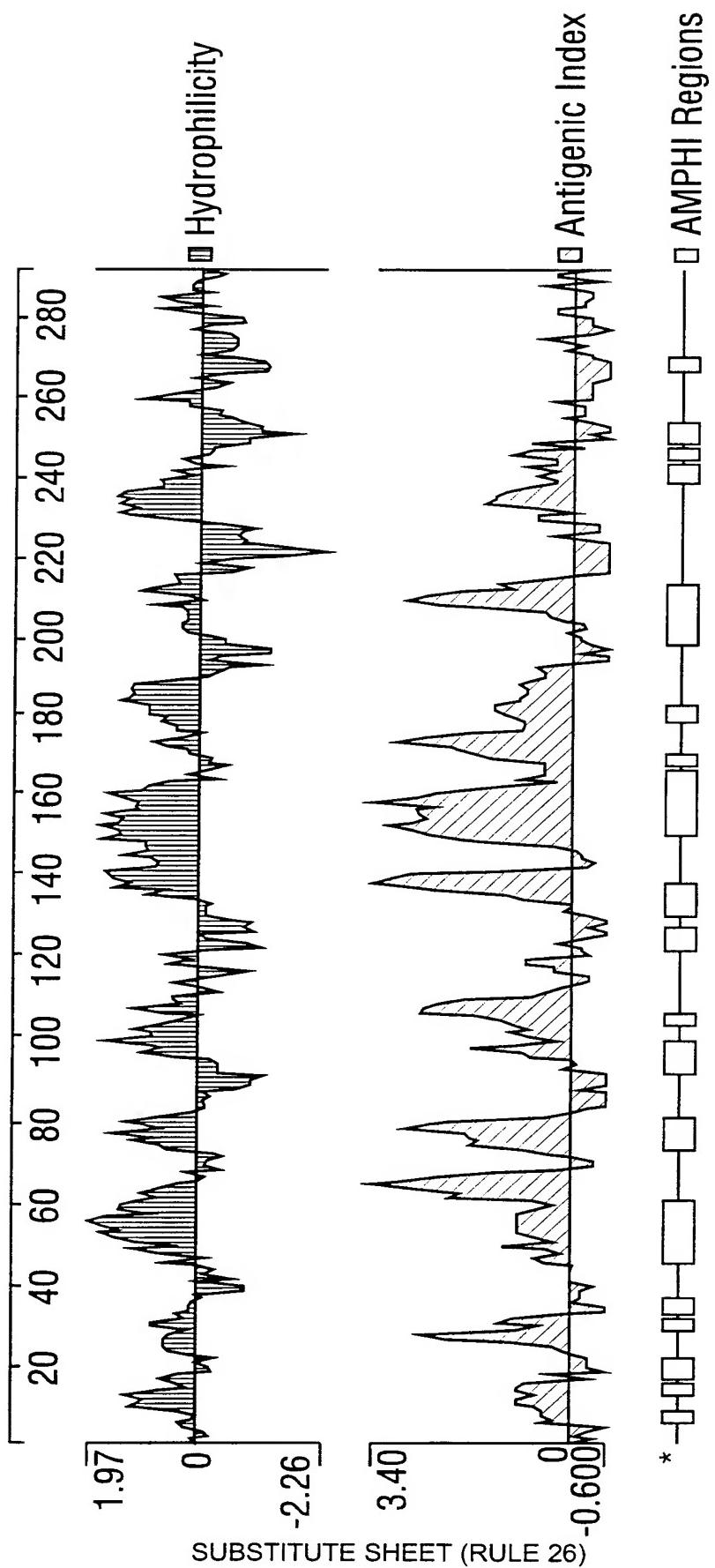


FIG. 21
ORF89



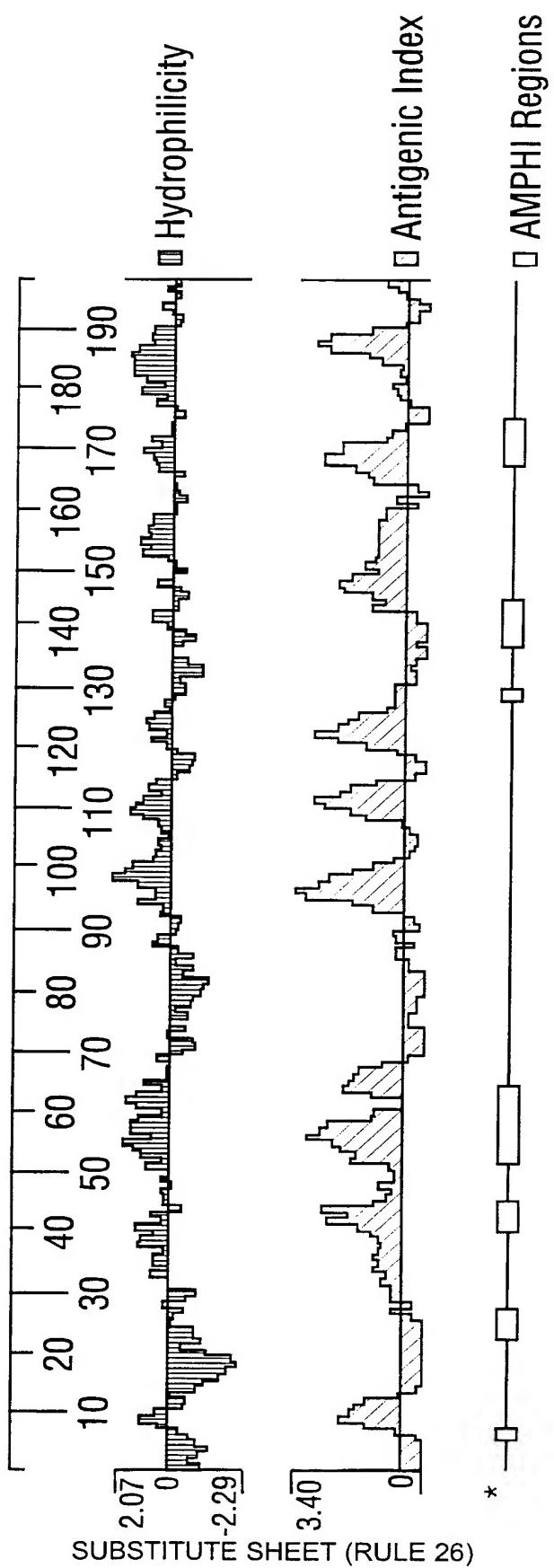
23/51

*FIG. 22
ORF105*



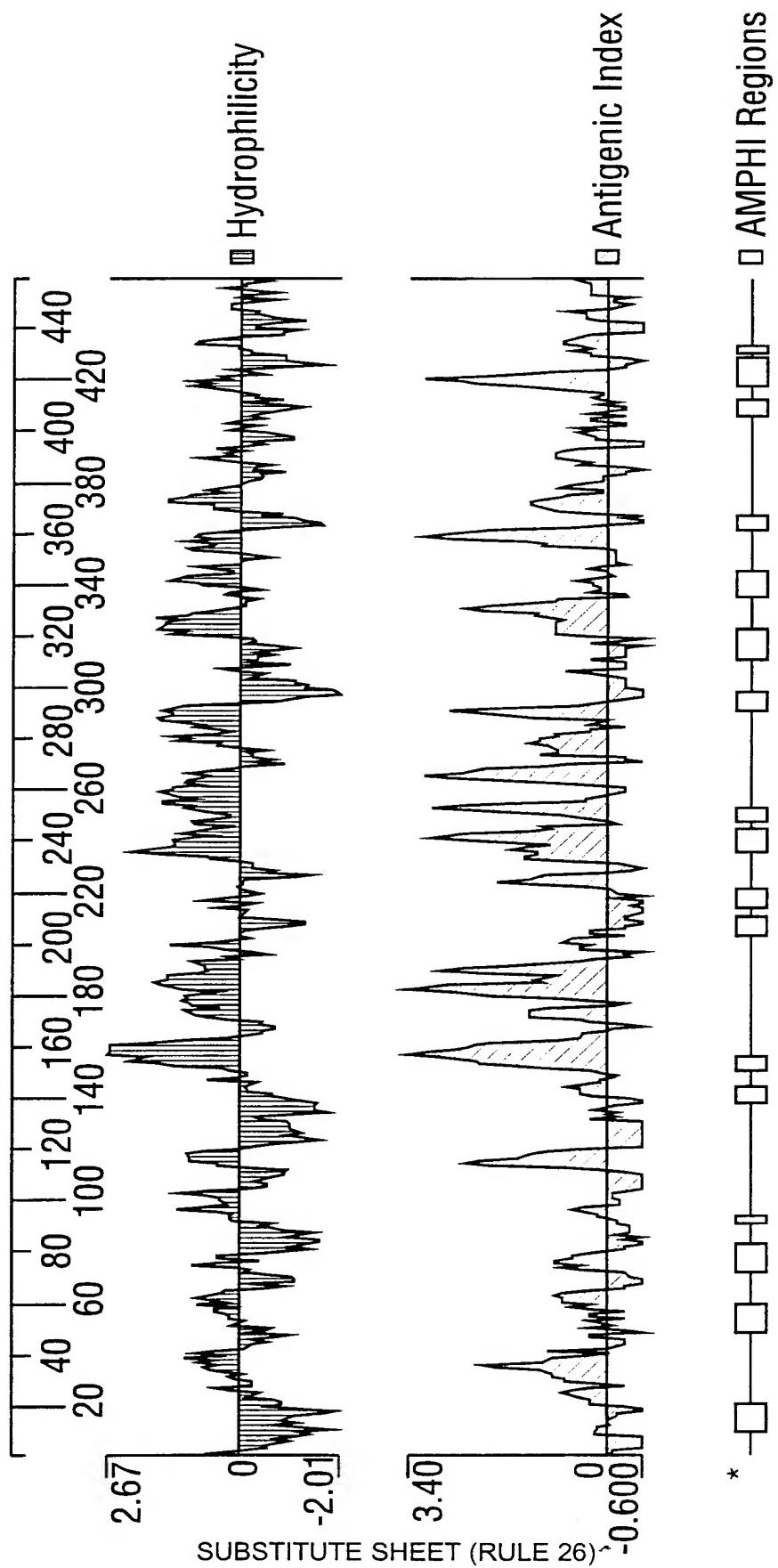
24/51

*FIG. 23
ORF106*



25/51

*FIG. 24
ORF132*



26/51

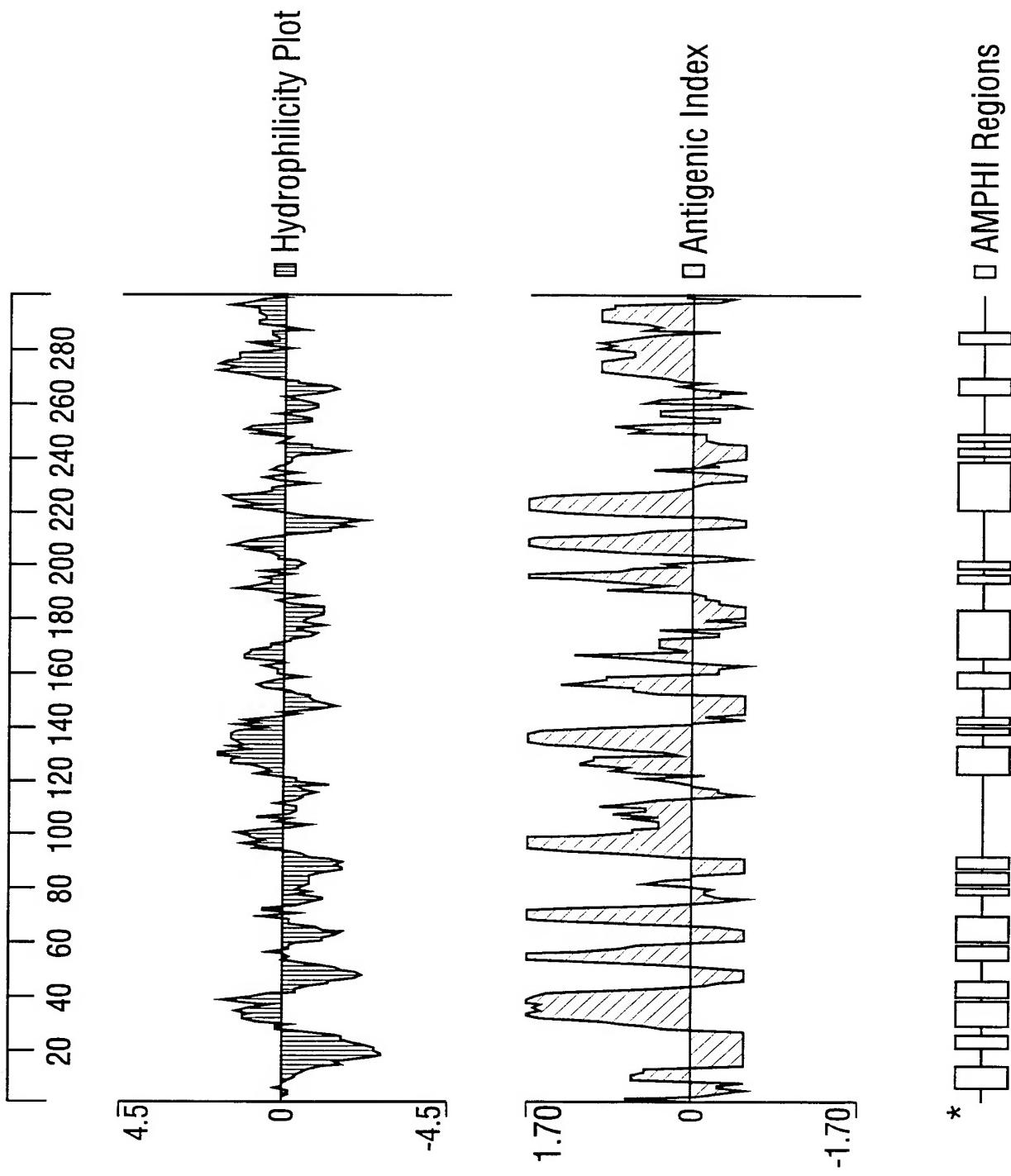
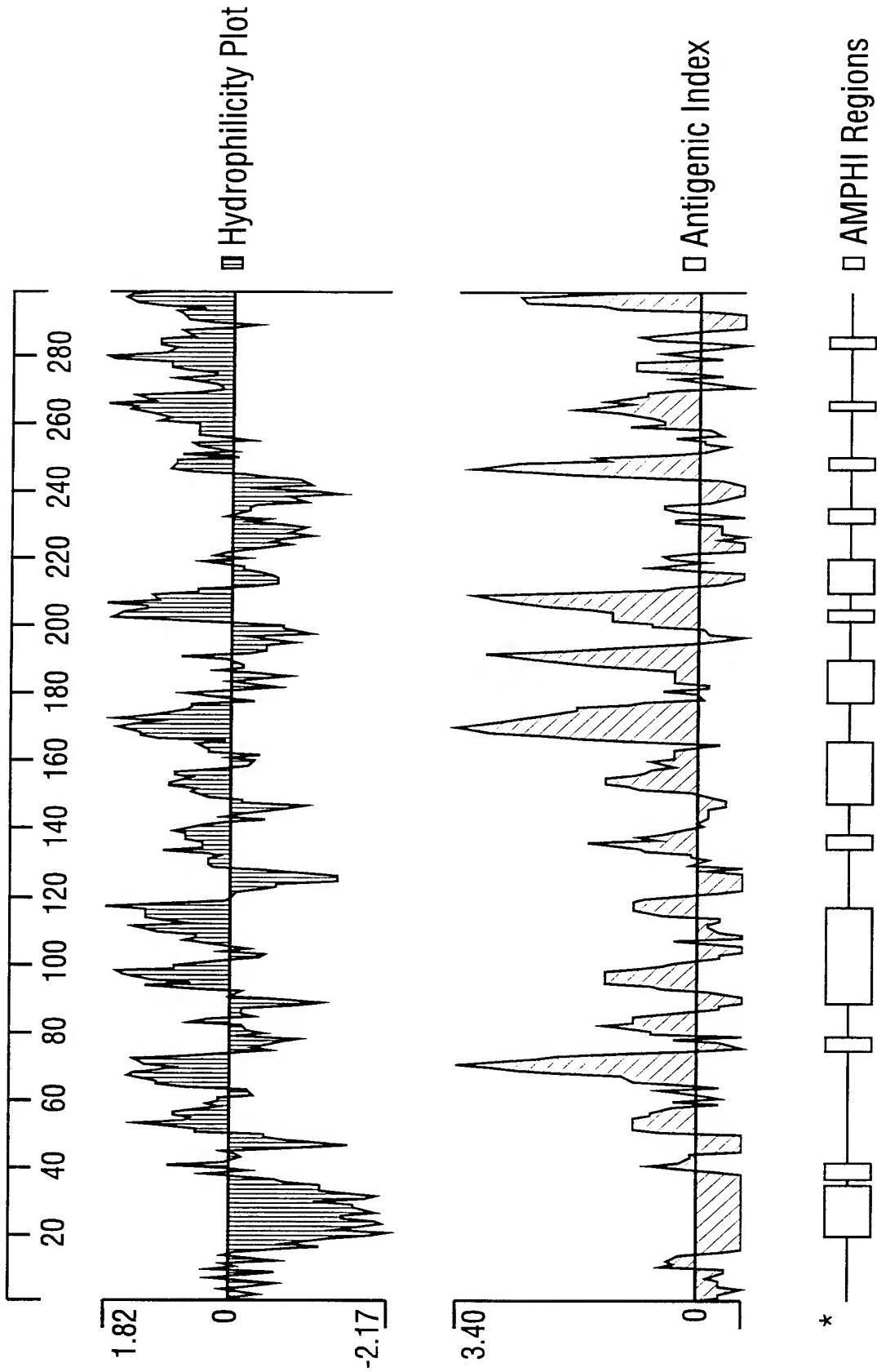


FIG. 25
ORF137

27/51

FIG. 26
ORF138



28/51

FIG. 27
ORF143

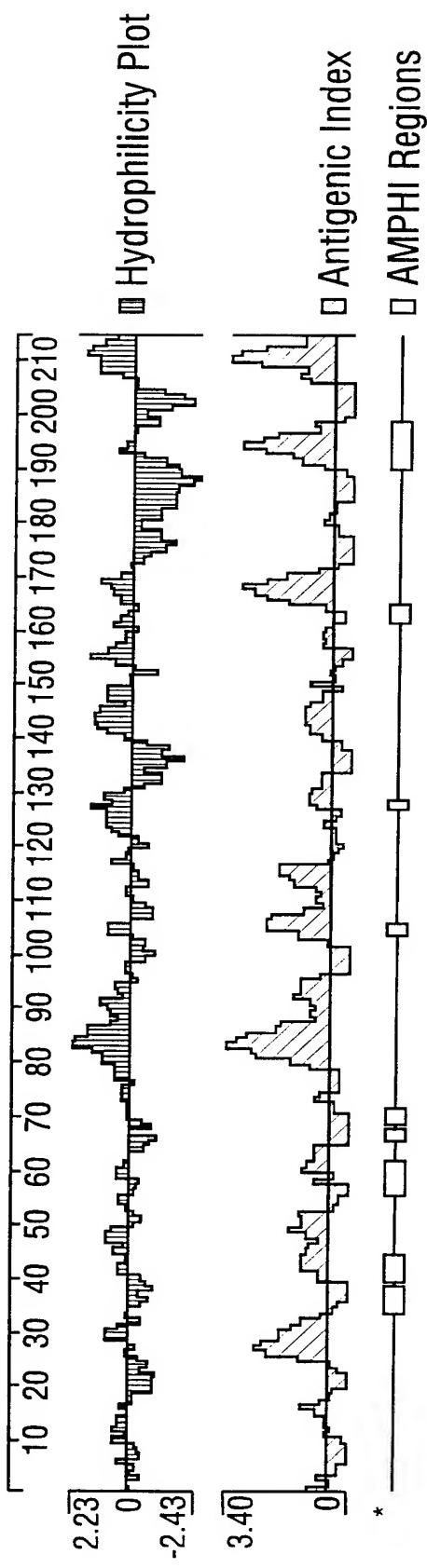
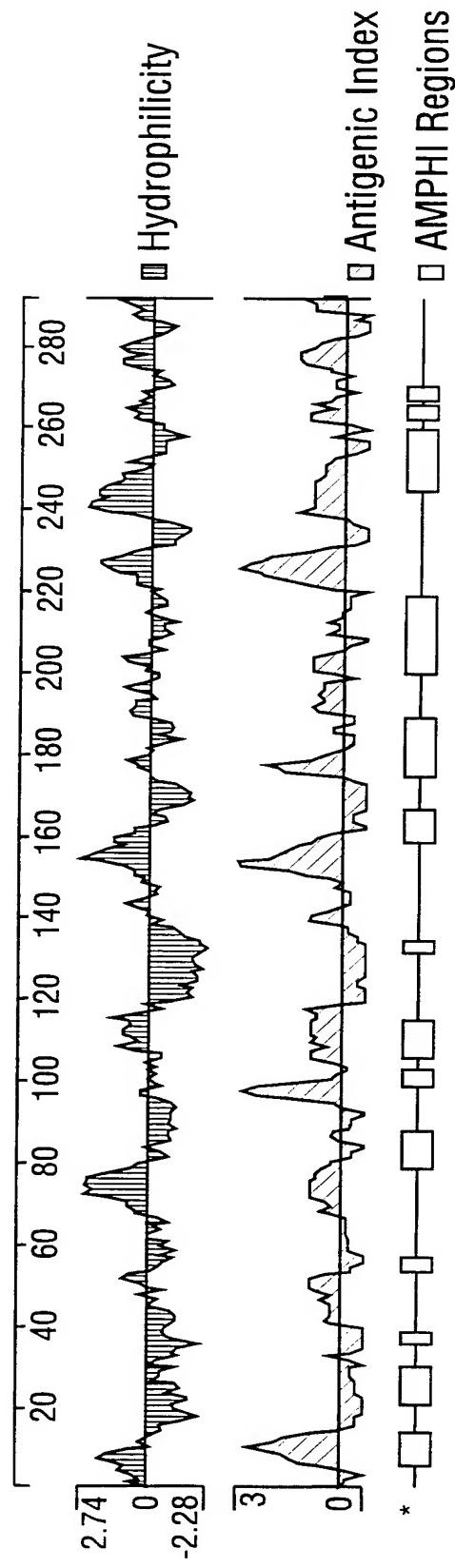


FIG. 28
ORF147



29/51

FIG. 29
NspA sequences

	Signal Peptide	Loop 1			
		-10	1	10	20
NM 608 B	MKKALATLIA LAPAAALAE GASGFYVQAD AAHAKASSL <u>GSAKGFSPRI</u> SAGYRINDL				
NM NG6/88+	-----	-----	-----	-----	-----
NM CU385+	-----	-----	-----	-----	-----
NM 8047+	----- A -----	-----	-----	-----	-----
NM NGP165-	-----	-----	-----	-----	-----
NM MC58-	-----	-----	-----	-----	-----
NM M986-	-----	-----	-----	-----	-----
NM BZ232-	----- I -----	-----	-----	-----	-----
NM M136-	-----	----- I -----	-----	-----	-----
NM NG3/88-	-----	----- I -----	-----	-----	-----
NG FA1090	----- A -----	-----	----- *	-----	-----
NG B2	----- A -----	-----	-----	-----	-----

30/51

FIG. 29 (contd.)
NspA sequences

		50	60	70	80	90	100	Loop 3
		RFAVDYTRYK	NYKAPSTDFK	LYSIGASAIY	DFDTQSPVVP	YLIGARLSLN	ASVDLGGSDS	
NM	608B							
NM	NG6/88+							
NM	CU385+							
NM	8047+							
NM	NGP165-							
NM	MC58-							
NM	M986-							
NM	BZ232-							
NM	M136-							
NM	NG3/88-							
NG	FA1090							
NG	B2							

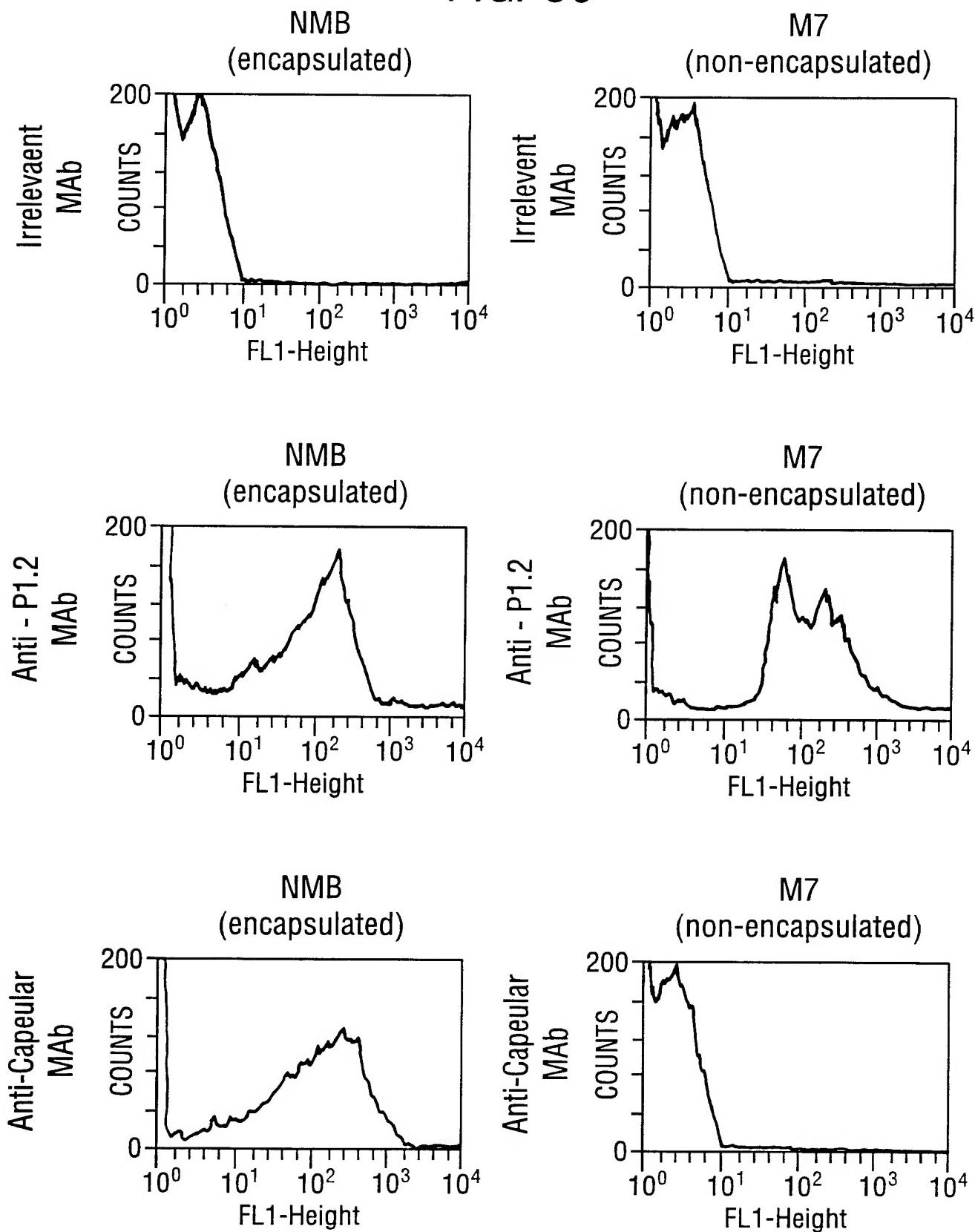
31/51

		110	120	130	140	150	Loop 4
NM	608B	<u>FSQTSIGLGV</u>	<u>LTVGSYAVTP</u>	<u>NVDLDAGYRY</u>	<u>NYIGKVNNTVK</u>	<u>NVRSGELSVG</u>	<u>VRVKF</u>
NM	NG6/88+	-	-	-	-	-	-
NM	8047+	-	-	T	-	A	-
NM	CU385+	-	-	-	-	-	A
NM	NGP165-	-	-	-	-	-	-
NM	MC58-	-	-	-	-	-	A
NM	M986-	-	-	-	-	-	-
NM	BZ232-	-	-	T	-	-	A
NM	M136-	-	-	T	-	A	-
NM	NG3/88-	-	-	T	-	A	-
NG	FA1090	-	-	K	-	A	-
NG	B2	-	-	K	-	A	-

FIG. 29 (contd.)
Nspa sequences

32/51

FIG. 30



33/51

FIG. 30 (CONT'D.)

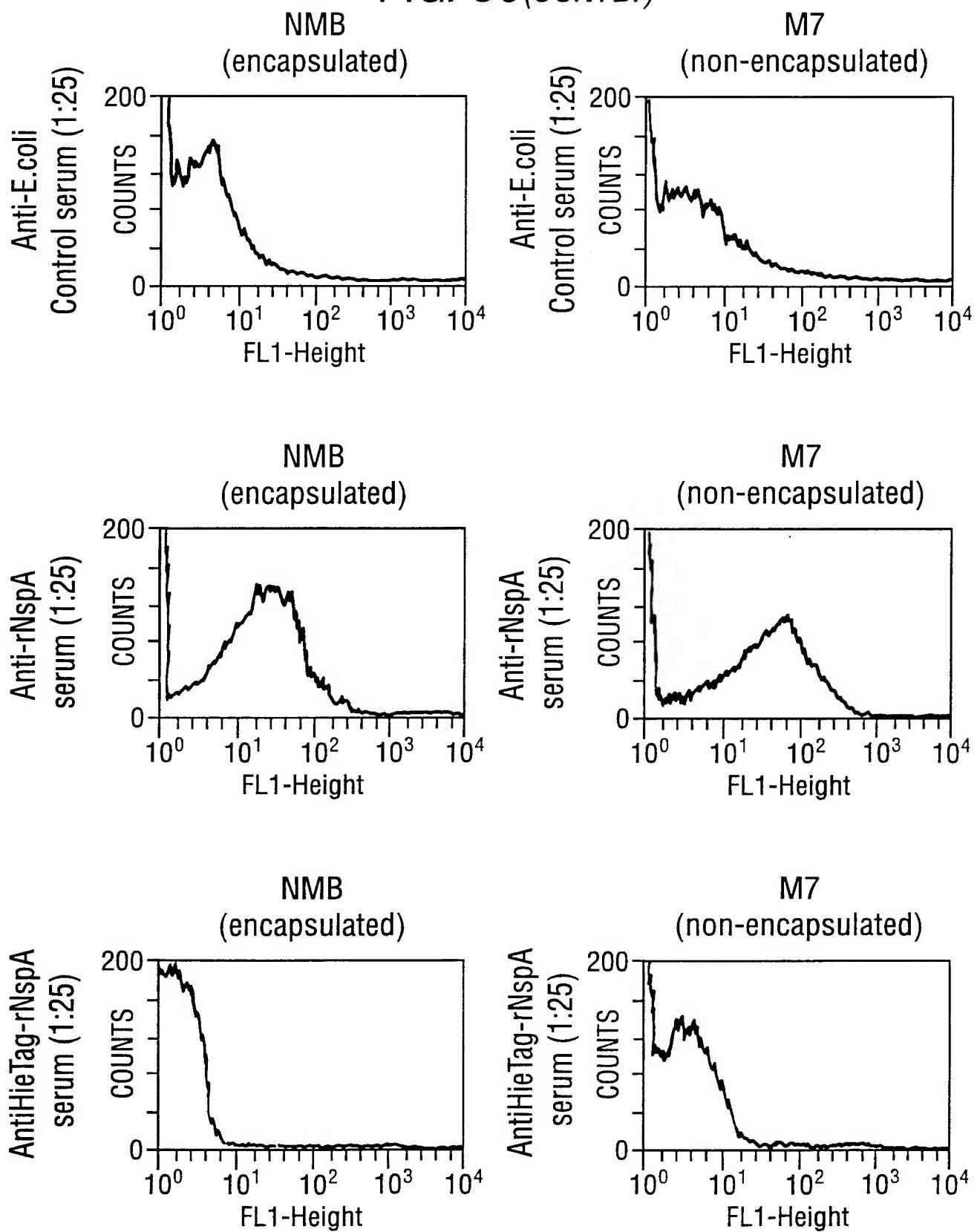


FIG. 31A

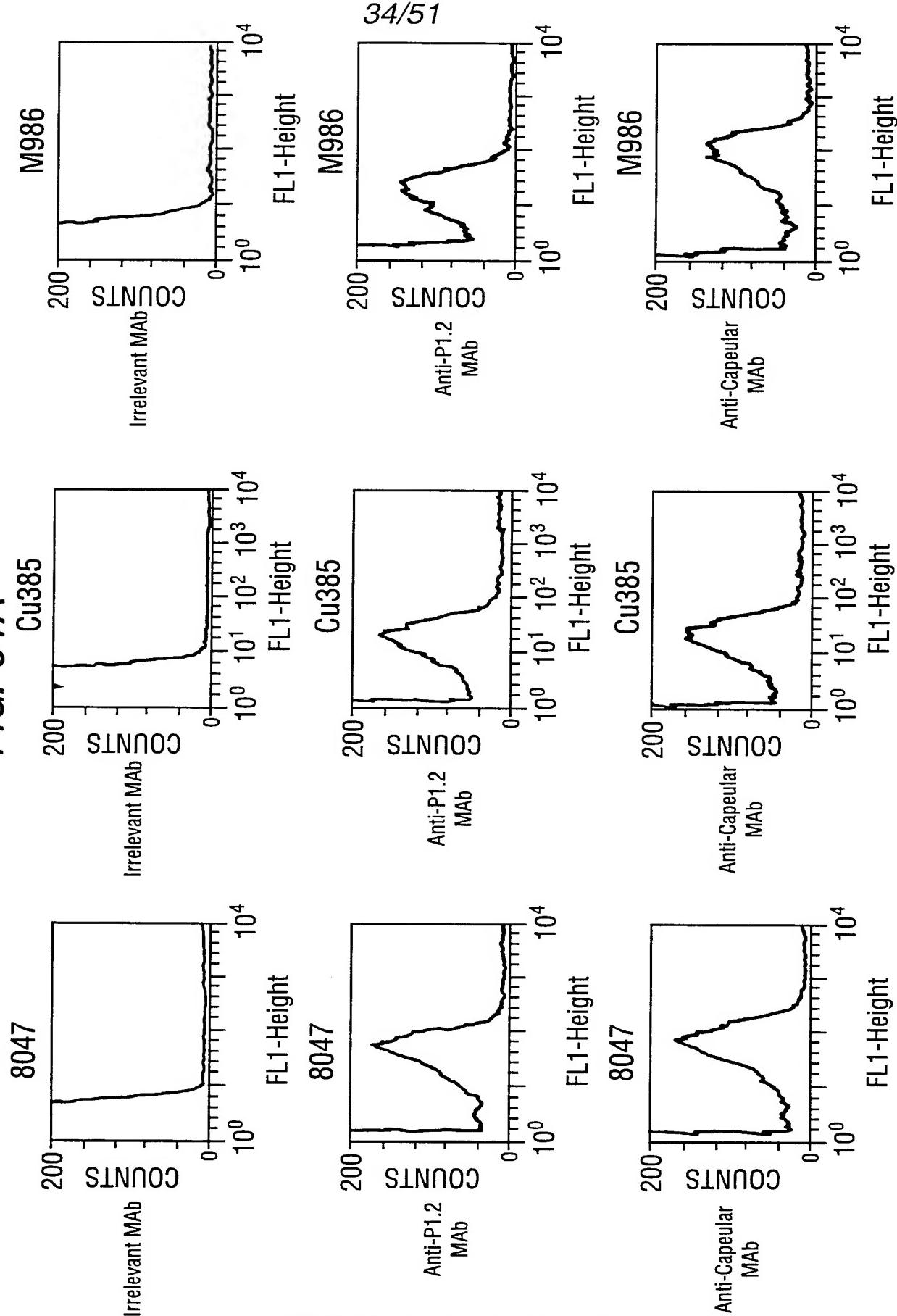


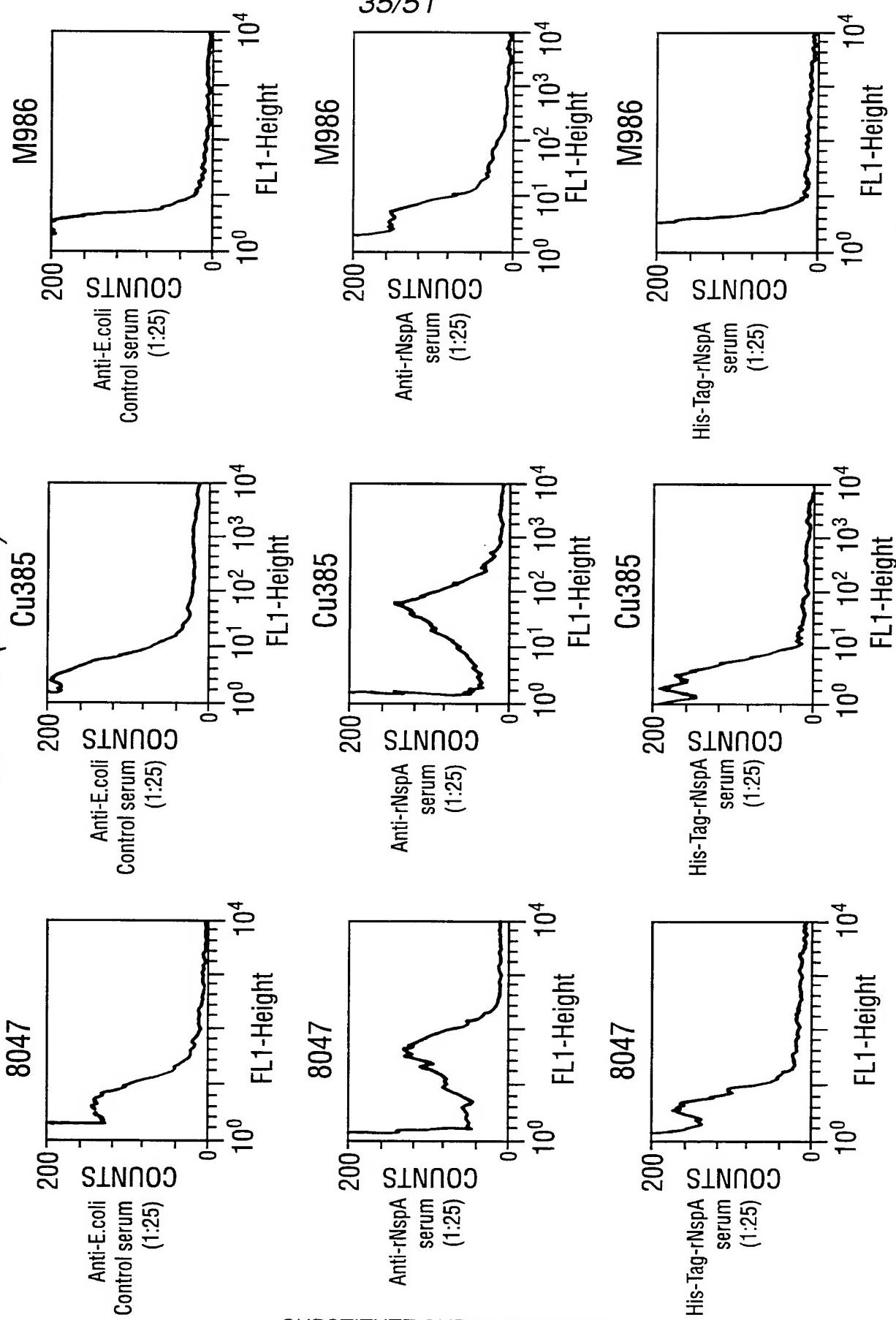
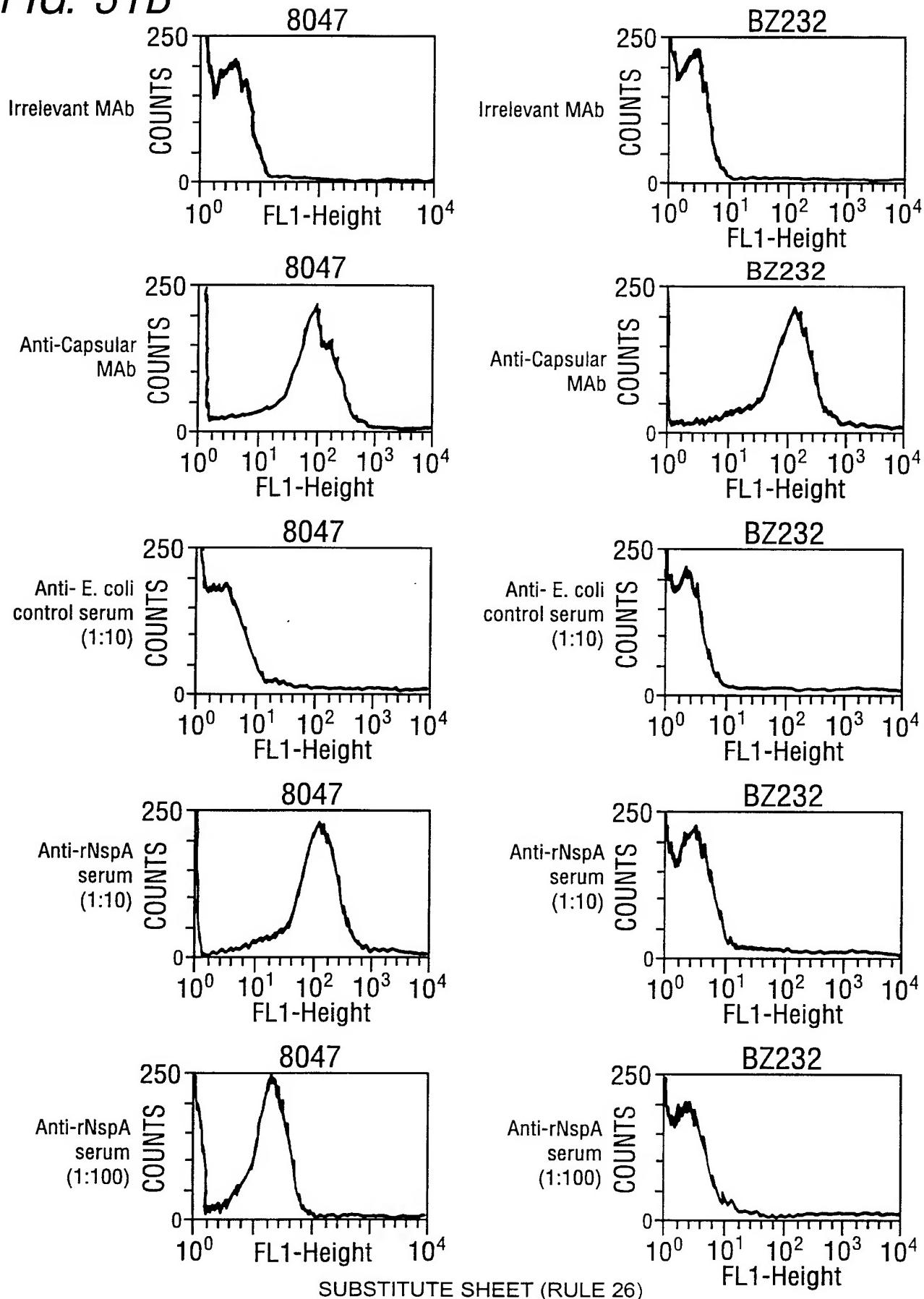
FIG. 31A (CONT'D.)

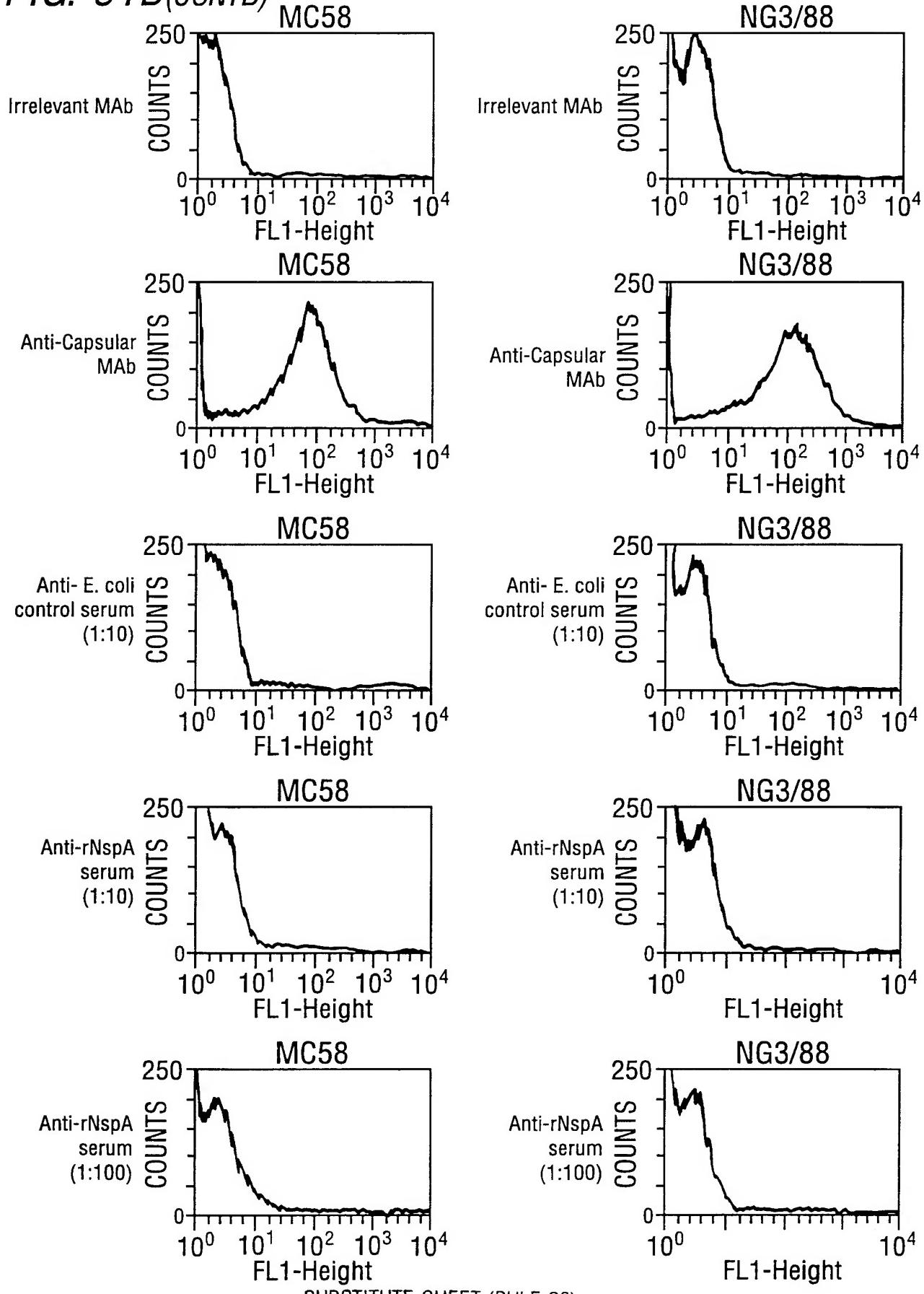
FIG. 31B

36/51



SUBSTITUTE SHEET (RULE 26)

FIG. 31B (CONT'D) 37/51



38/51

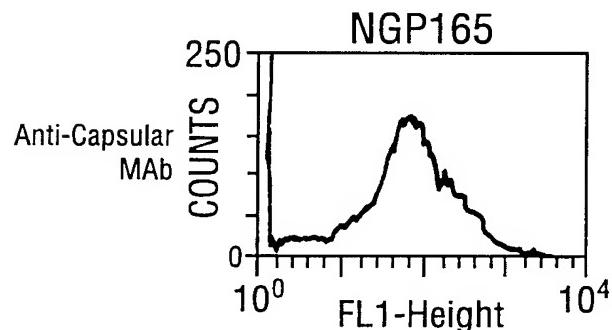
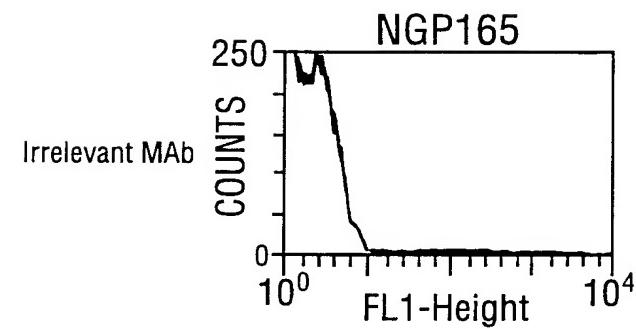


FIG. 31B(*CONTD*)

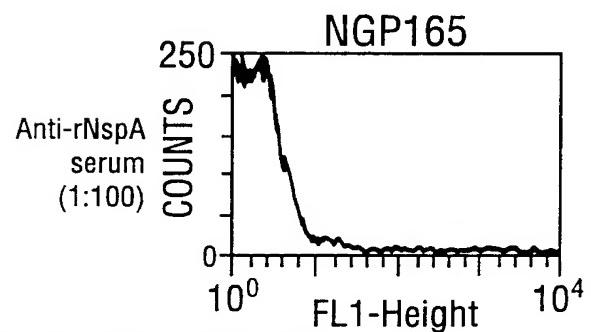
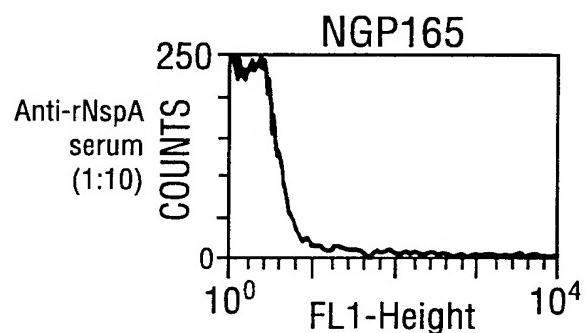
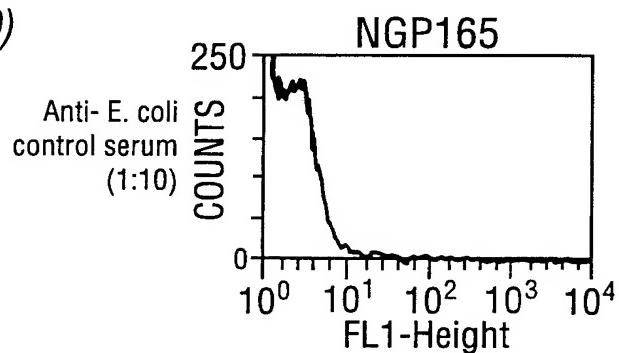
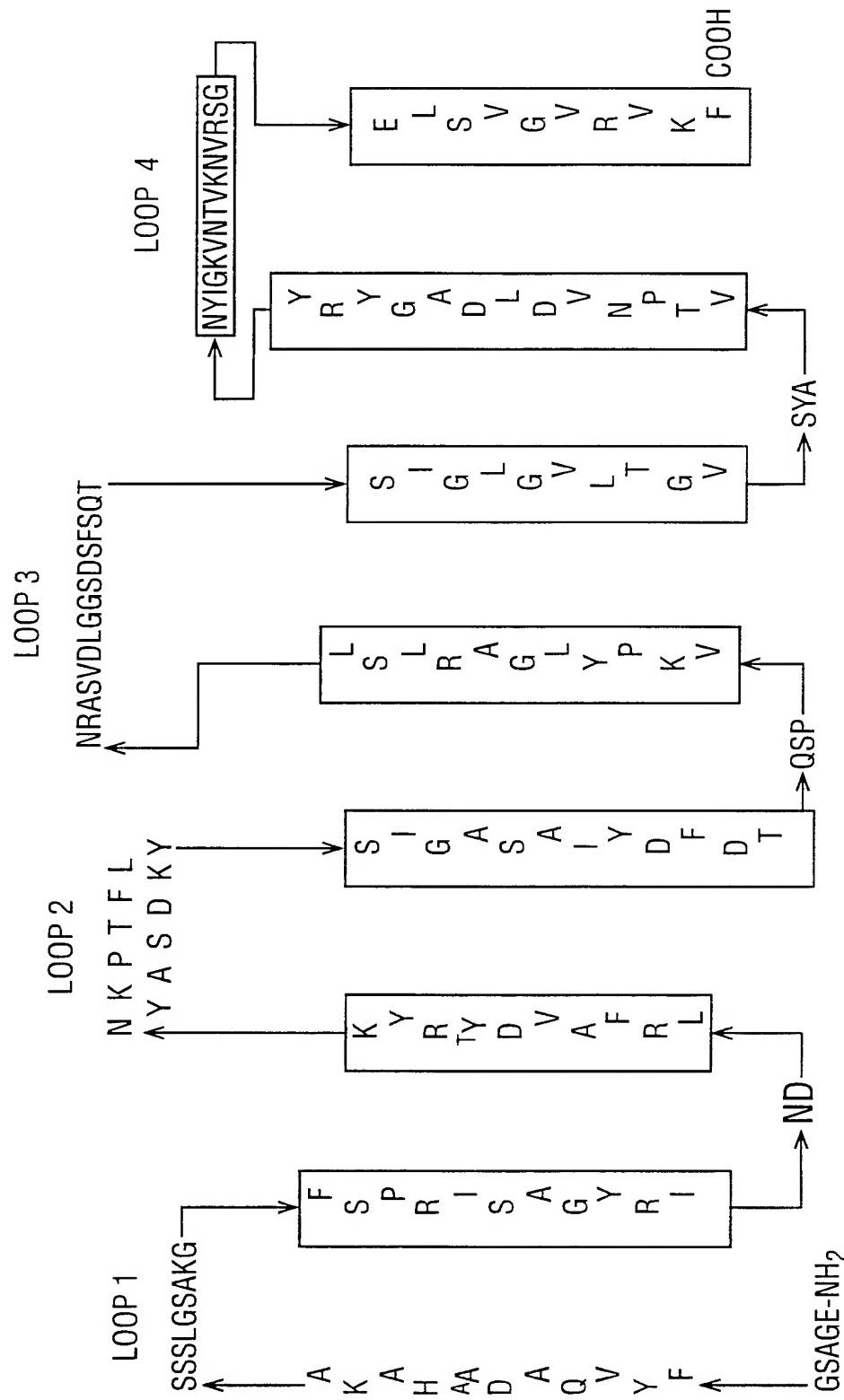
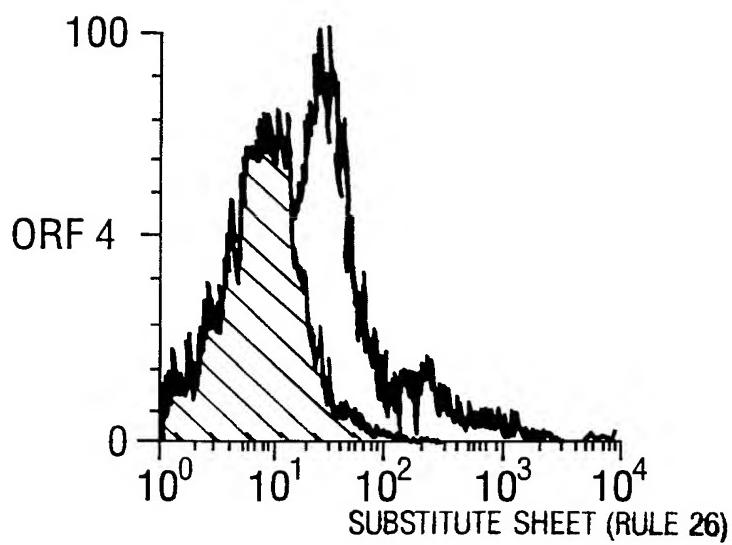
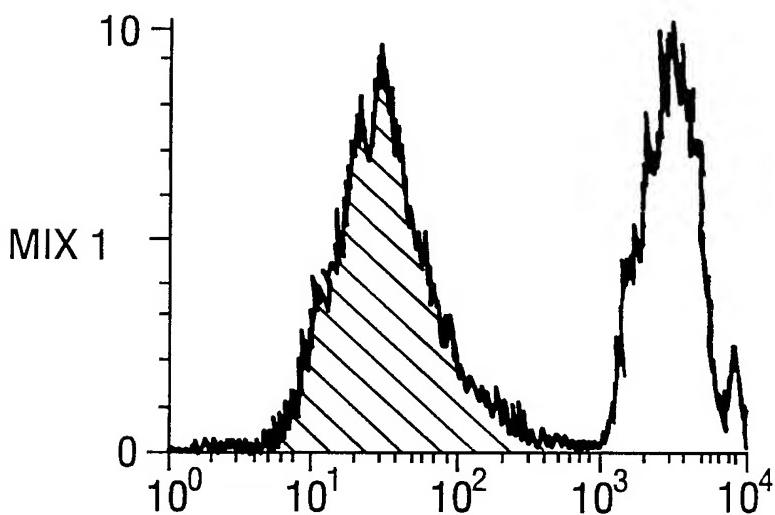
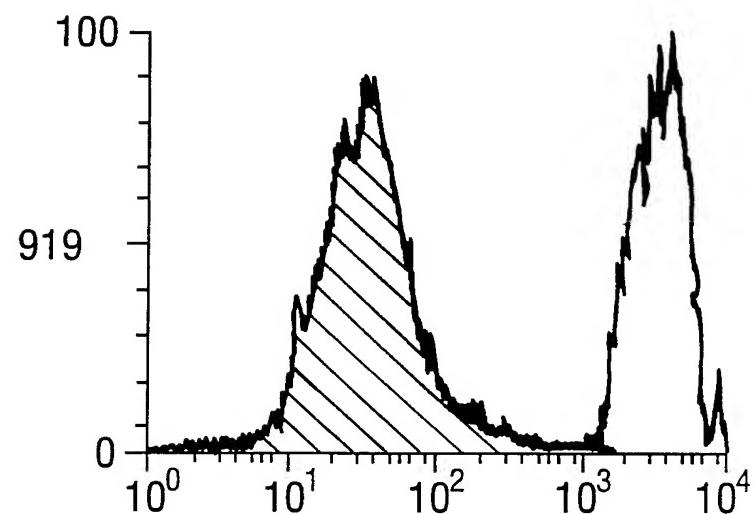


FIG. 32



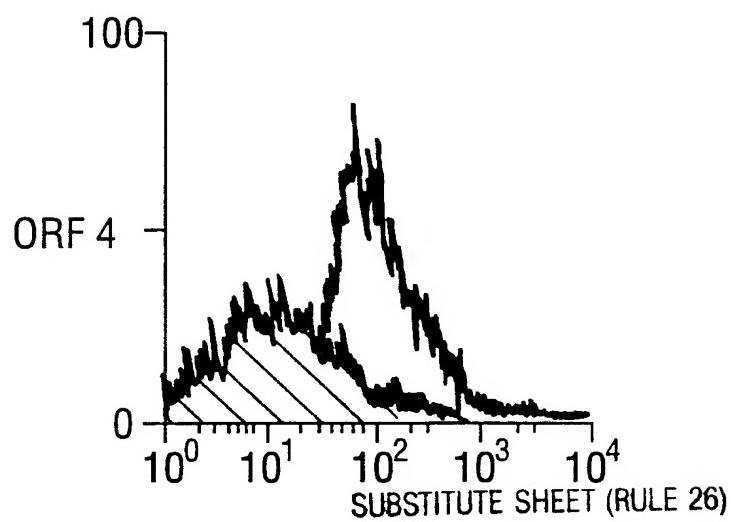
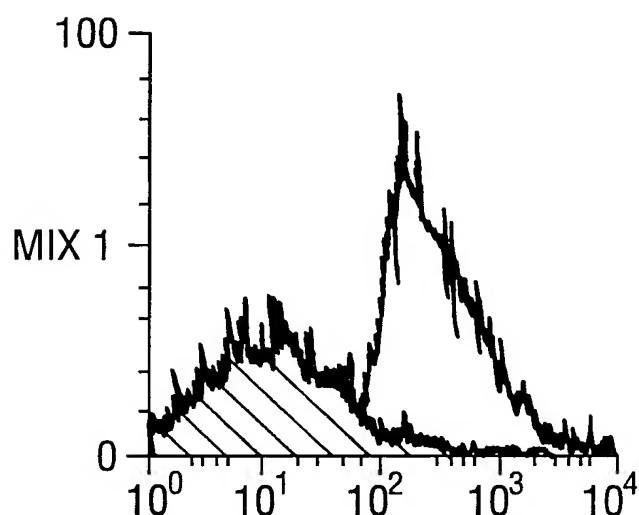
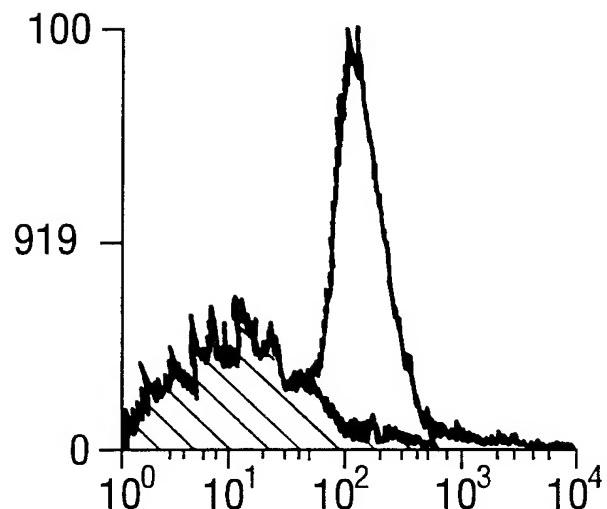
40/51

FIG. 33A

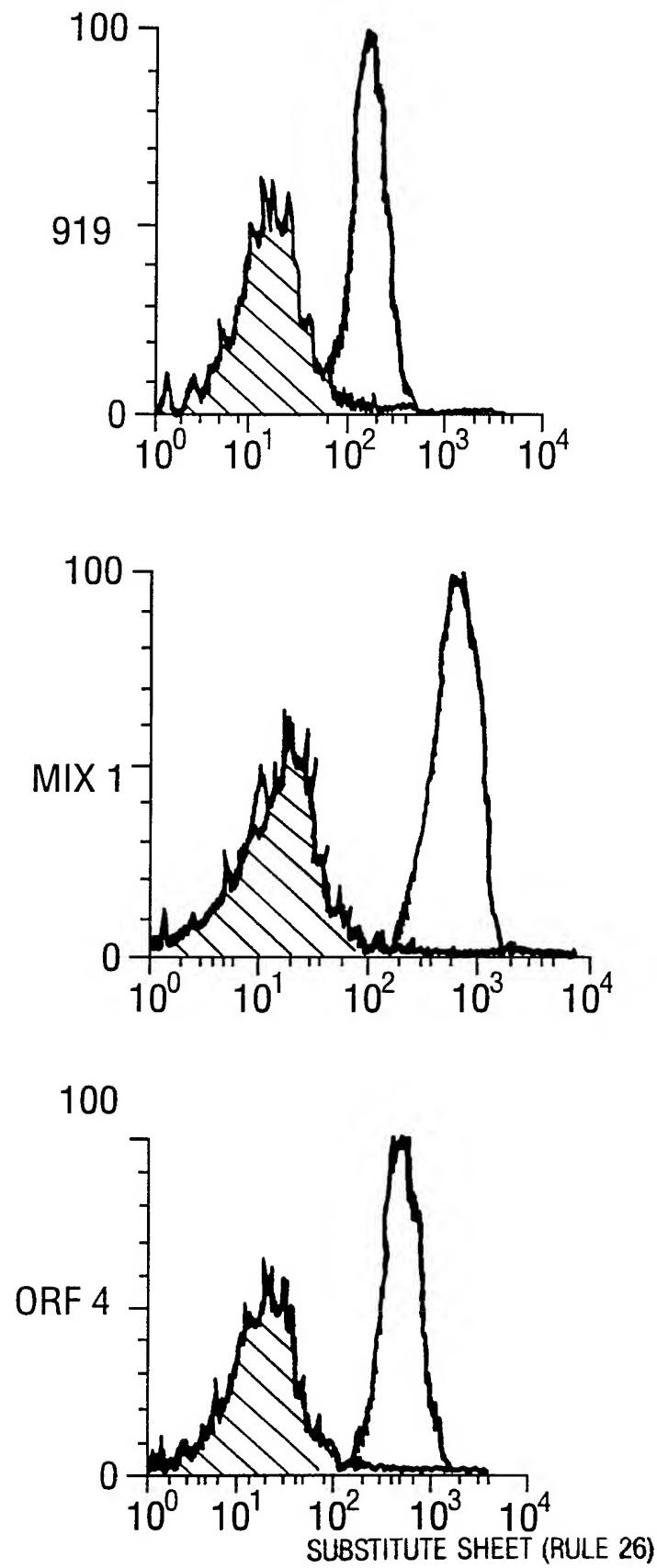


SUBSTITUTE SHEET (RULE 26)

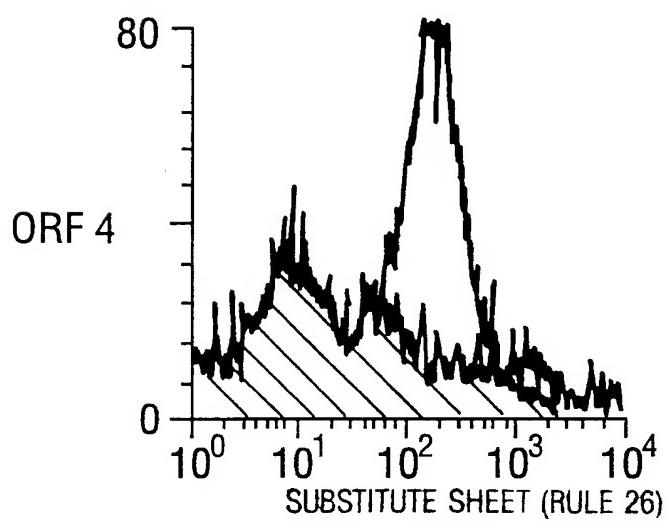
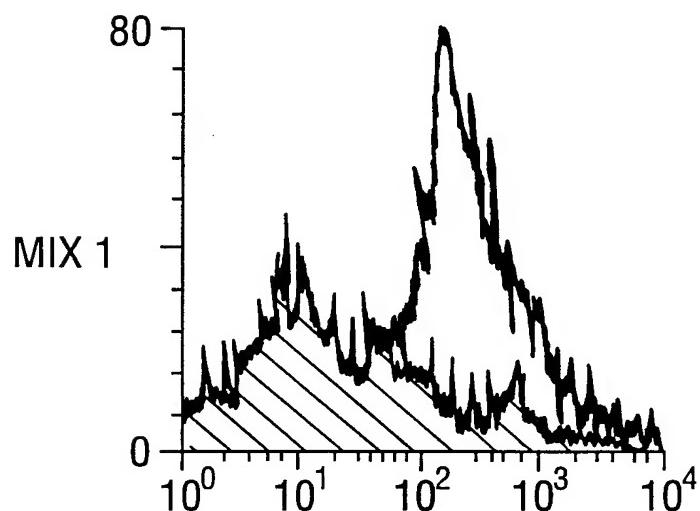
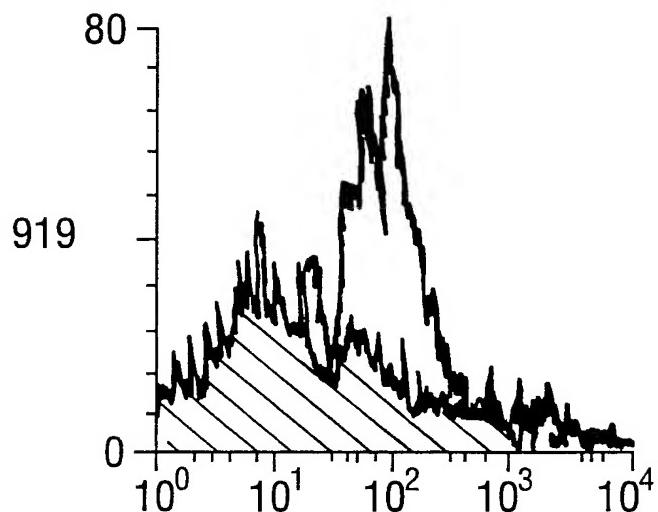
41/51
FIG. 33B
2996



42/51

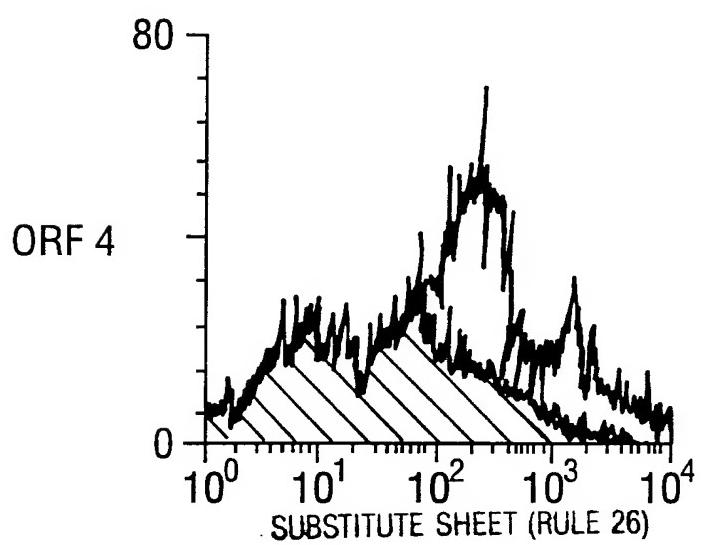
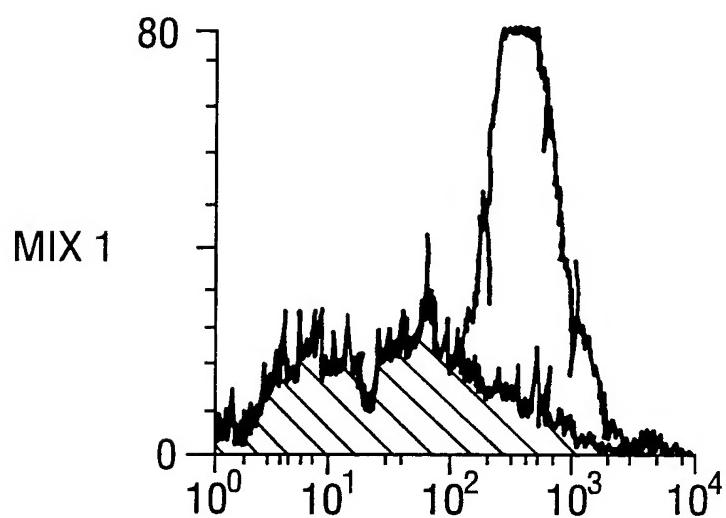
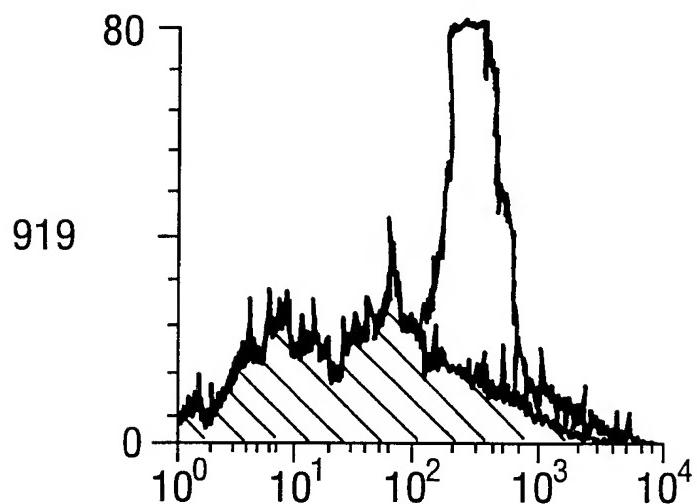
FIG. 33B (CONTD.)
H44776

43/51
FIG. 33B (CONTD.)
MC58



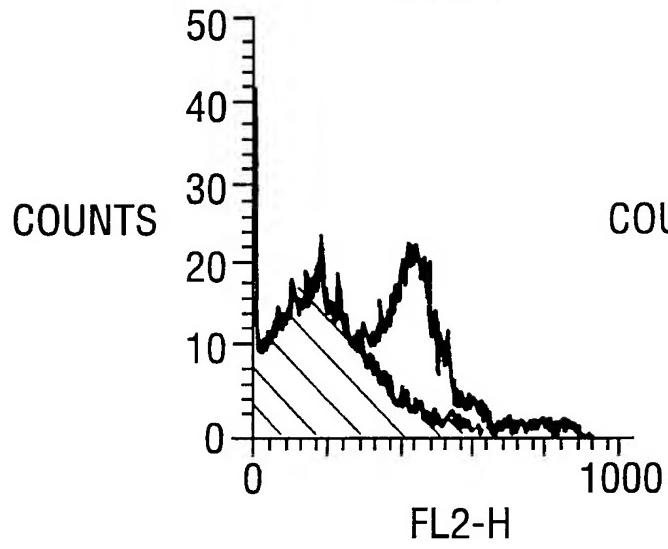
SUBSTITUTE SHEET (RULE 26)

44/51
FIG. 33B (CONTD.)
1000

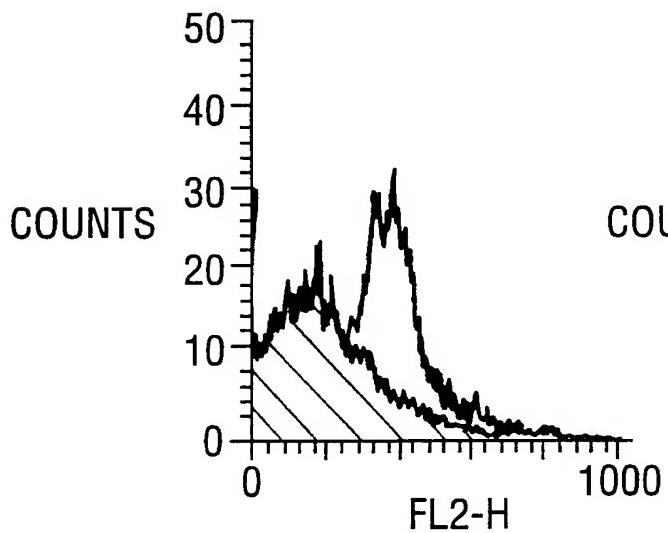
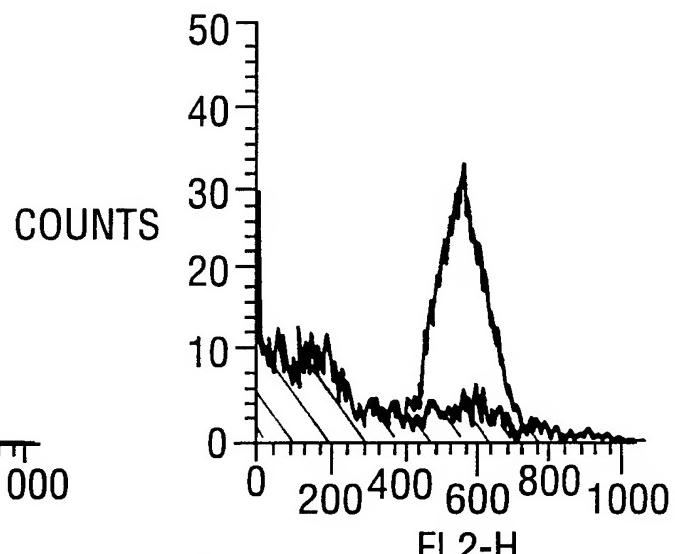
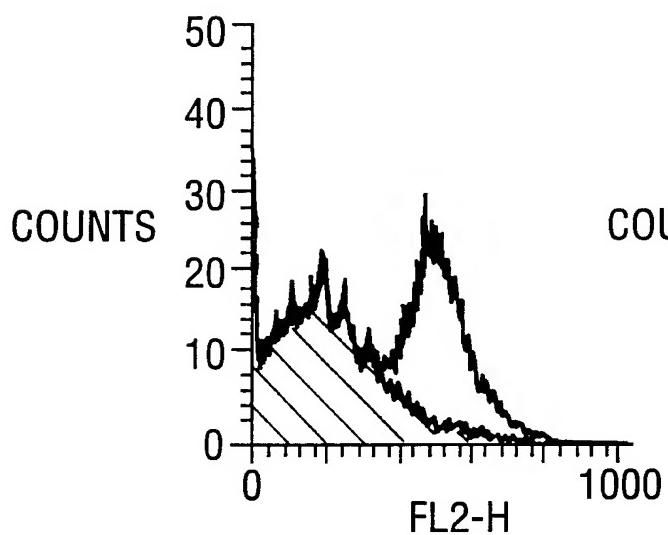
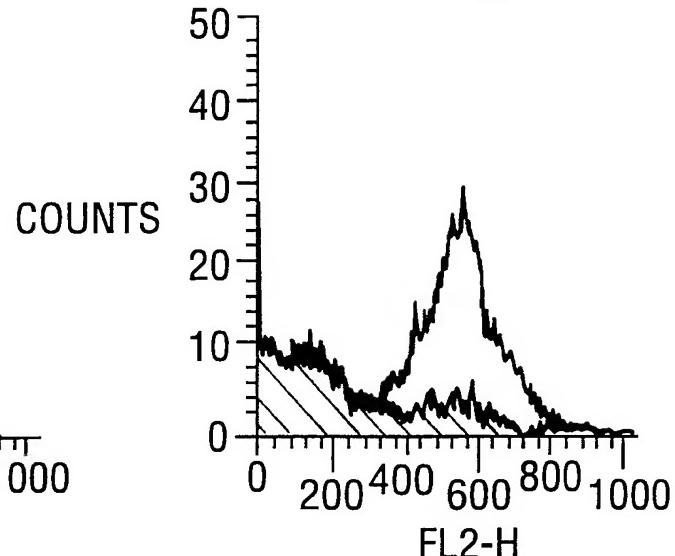


SUBSTITUTE SHEET (RULE 26)

45/51

FIG. 33B (CONT'D.)
BZ232

BZ133



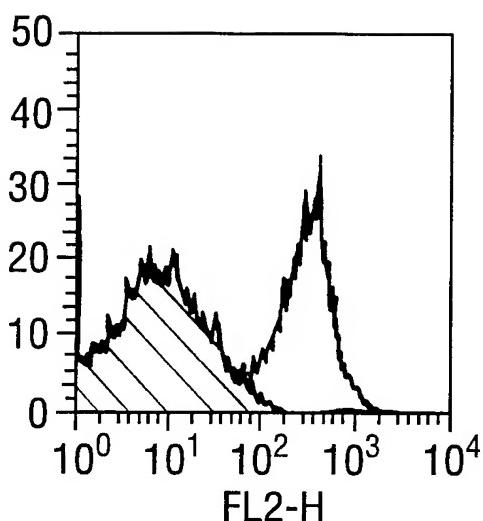
SUBSTITUTE SHEET (RULE 26)



46/51

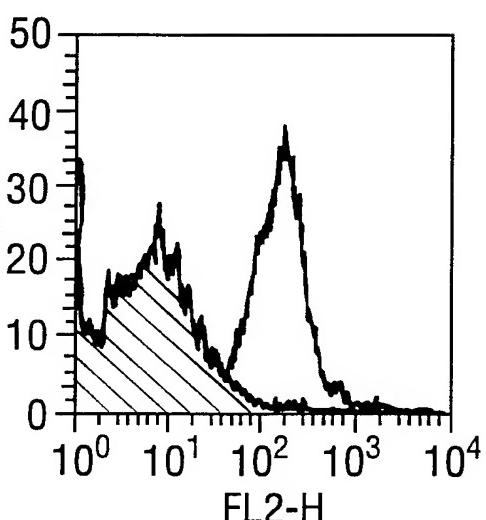
FIG. 33B (CONTD.)

NG 6/88

919
COUNTS

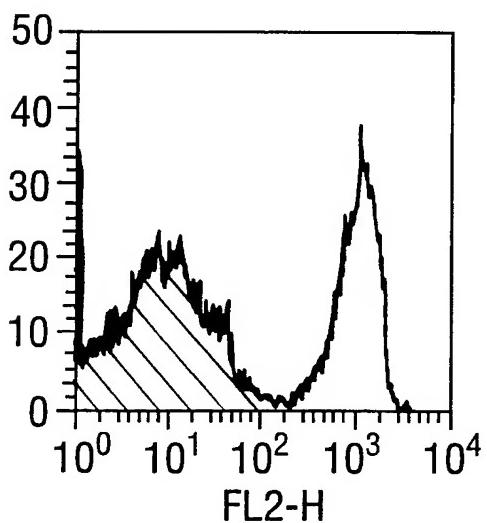
BZ 198

COUNTS

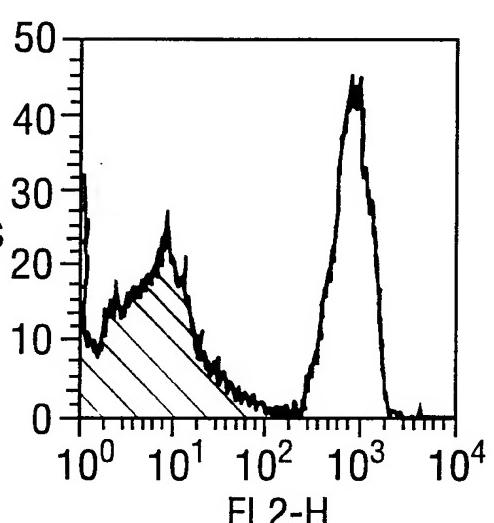


MIX 1

COUNTS

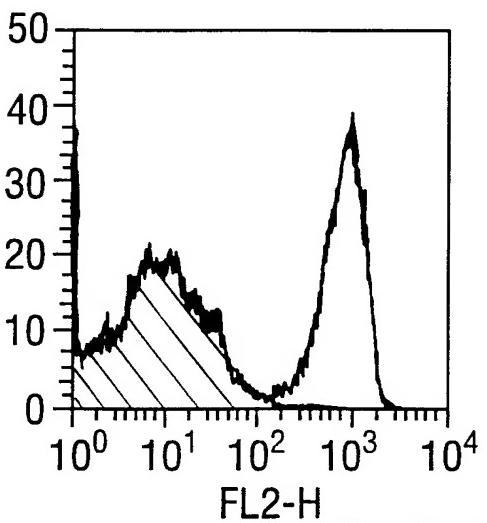


COUNTS

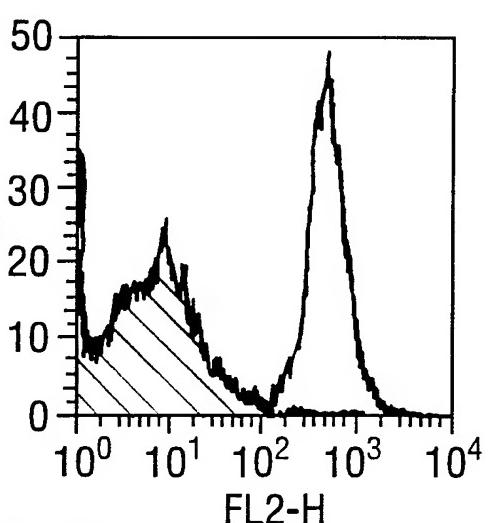


ORF4

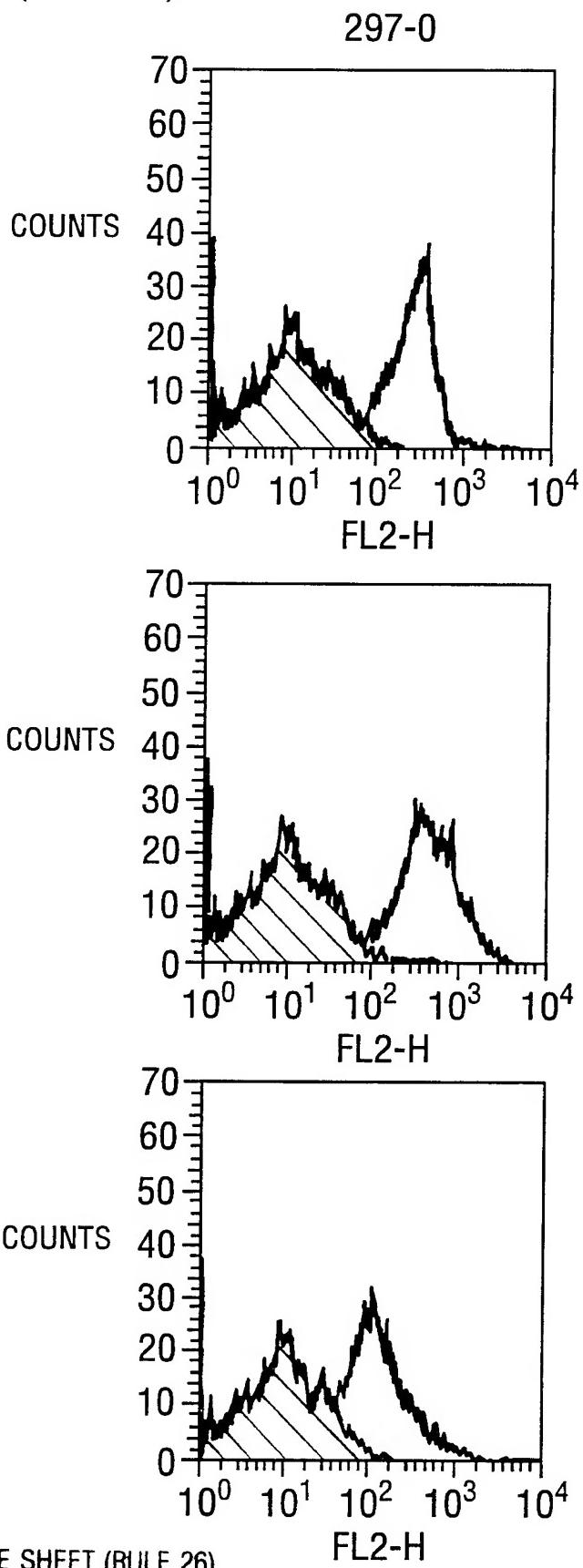
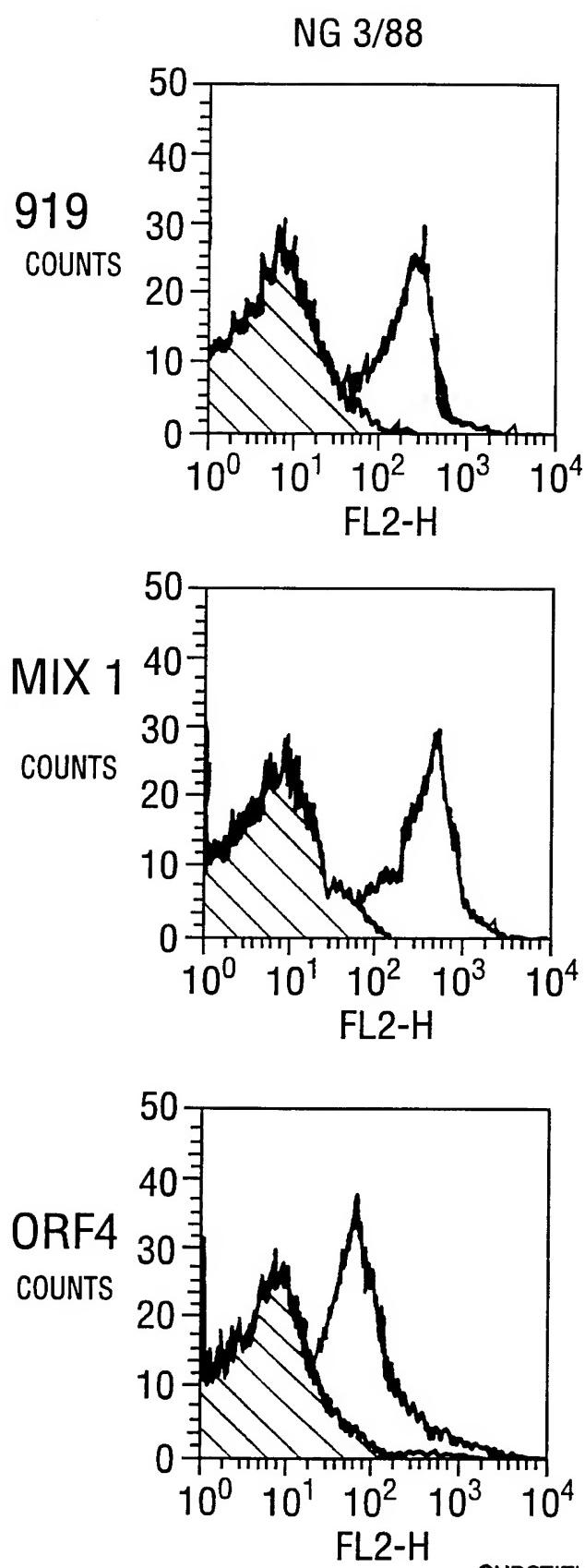
COUNTS



COUNTS



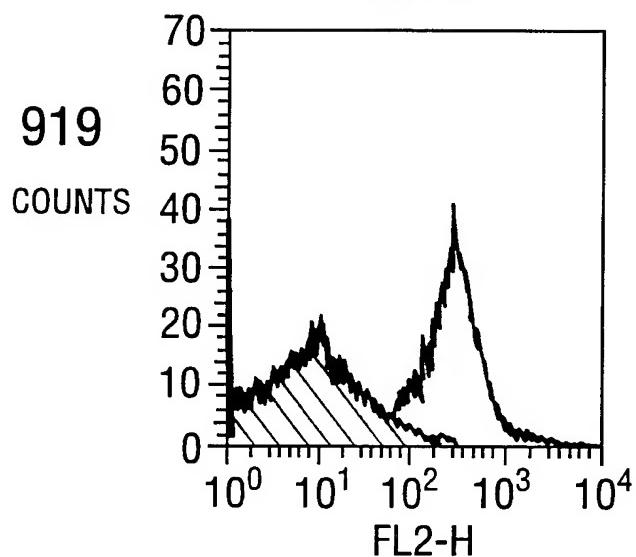
47/51

FIG. 33B (CONTD.)

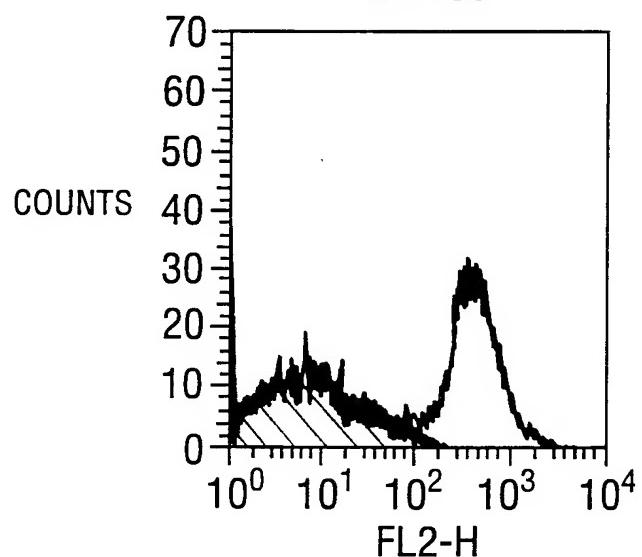
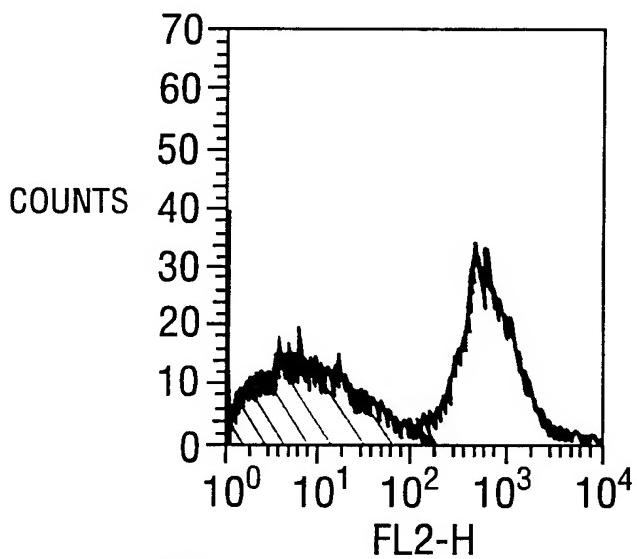
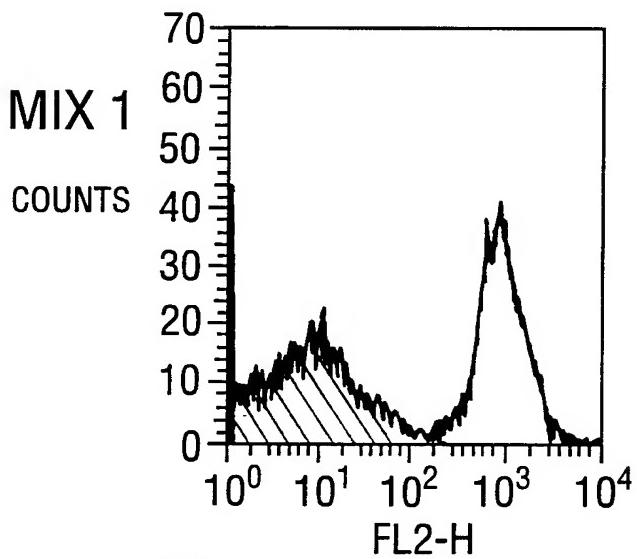
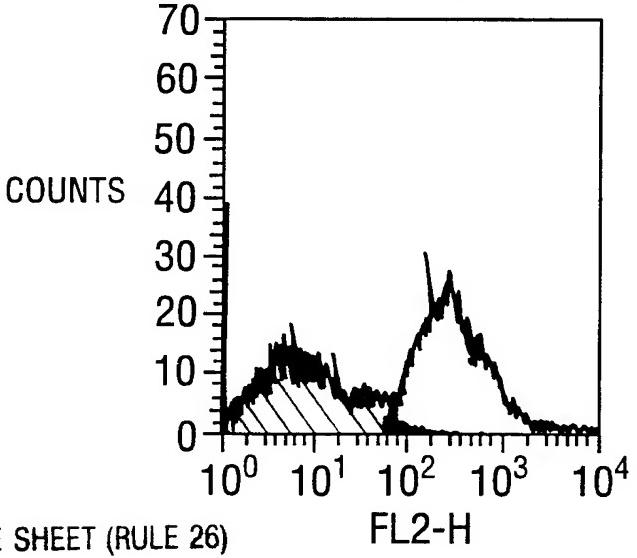
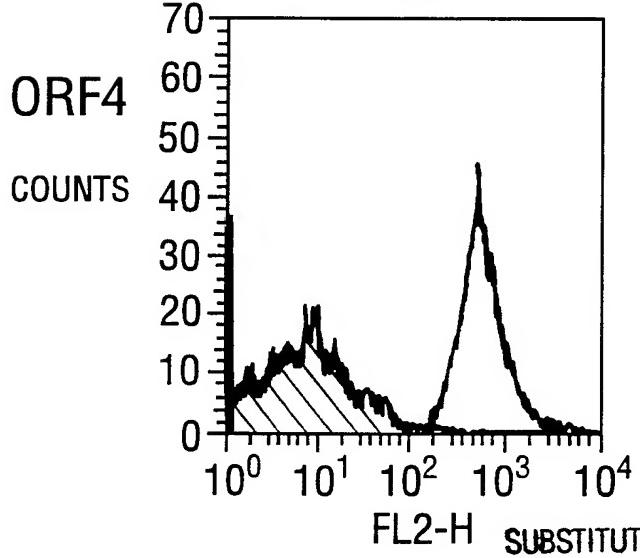
48/51

FIG. 33B (CONTD.)

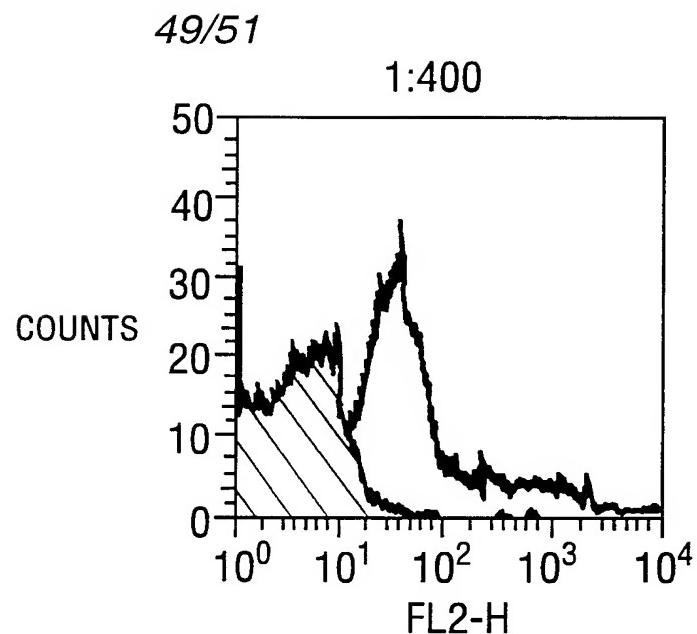
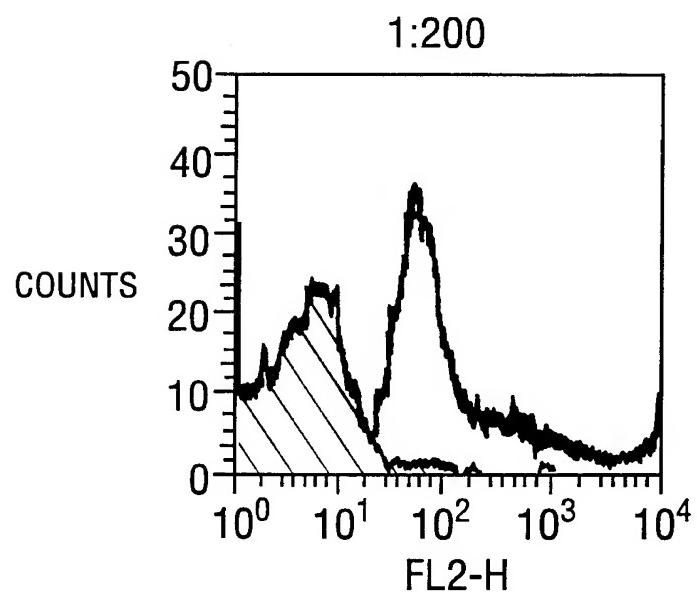
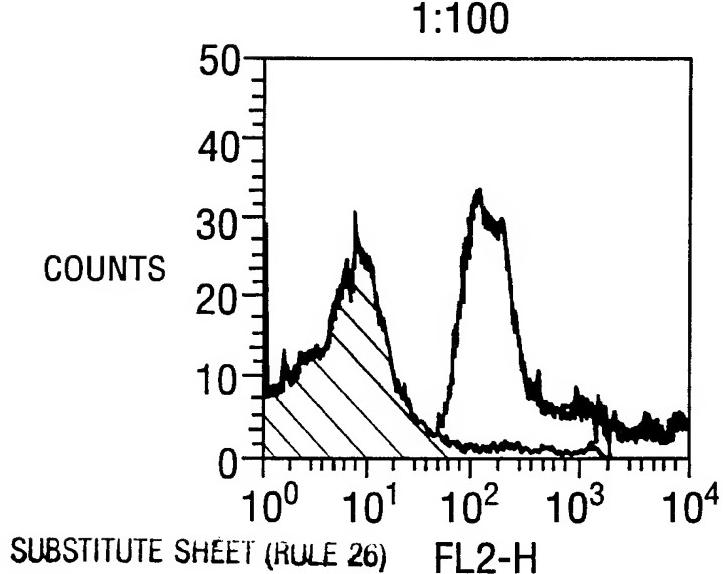
BZ147



BZ 169

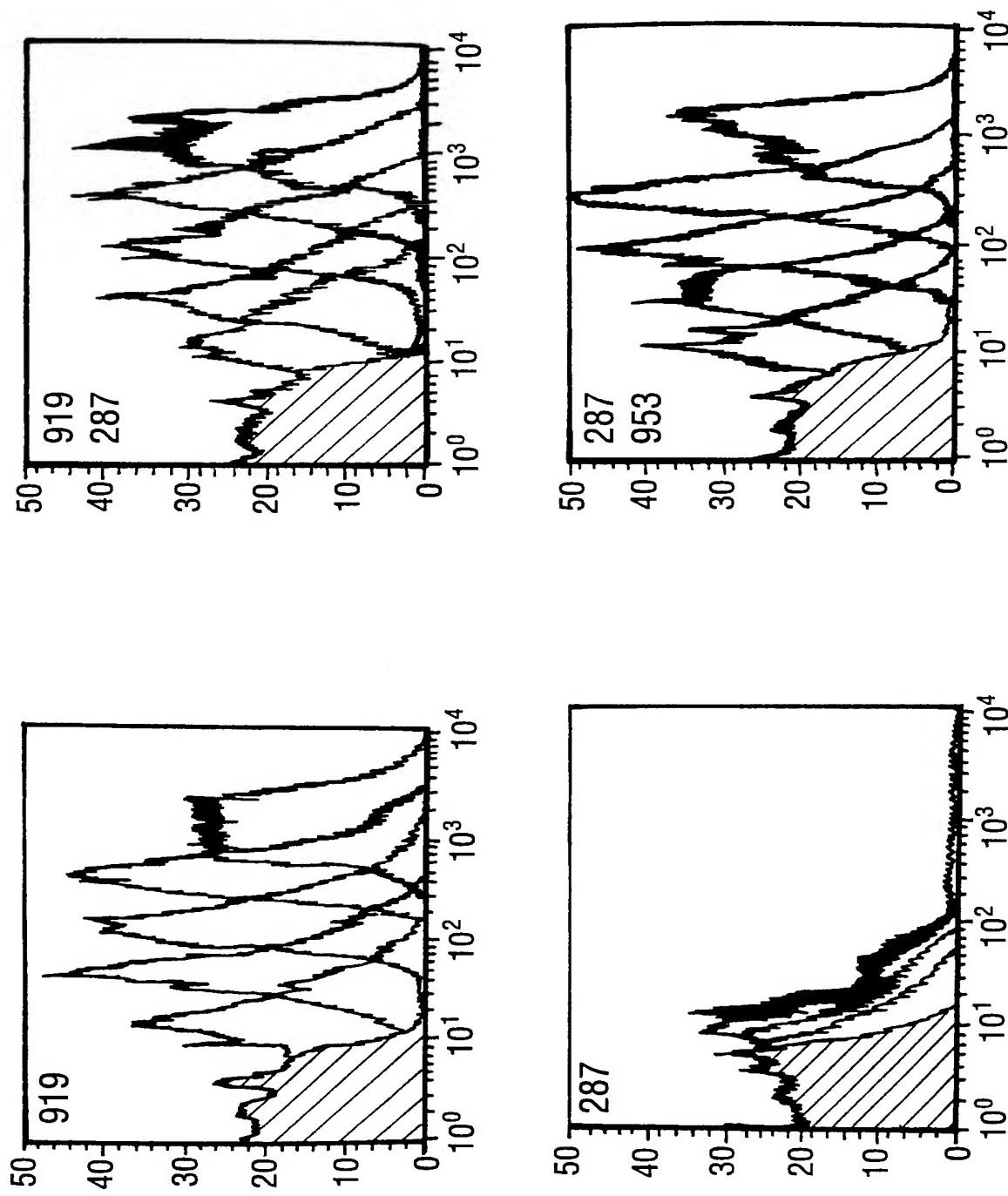
**MIX 1****ORF4**

SUBSTITUTE SHEET (RULE 26)

FIG. 34A*FIG. 34B**FIG. 34C*

50/51

FIG. 35



51/51

FIG. 35 (CONT'D.)

